The Institute of Paper Chemistry

Appleton, Wisconsin

Doctor's Dissertation

Synthesis and Alkaline Degradation of Xylobiose and 2', 3', 4'-Tri-*O*-Methyl-Xylobiose

James R. Kidd

January, 1980

SYNTHESIS AND ALKALINE DEGRADATION OF XYLOBIOSE AND 2',3',4'-TRI-O-METHYL-XYLOBIOSE

A thesis submitted by

James R. Kidd

B.S. 1973, University of Wisconsin-LaCrosse

M.S. 1976, Lawrence University

in partial fulfillment of the requirements
of The Institute of Paper Chemistry
for the degree of Doctor of Philosophy
from Lawrence University,
Appleton, Wisconsin

January, 1980

TABLE OF CONTENTS

SUMMARY

The objective of this research was to determine whether ionization of hydroxyl groups in the nonreducing glycose unit (leaving group) of a disaccharide affected the oxygen-free alkaline degradation of a reducing disaccharide. The study involved the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose in 0.1 and 2.5M aqueous sodium hydroxide. The hydroxyl groups in the glycose unit of xylobiose are free to ionize, whereas, in 2',3',4'-tri-O-methyl-xylobiose these positions are etherified. Therefore, effects of ionization of hydroxyl groups in the nonreducing glycose unit would be manifested in differences in degradation rates or products for the two model compounds.

Xylobiose and 2',3',4'-tri-O-methyl-xylobiose were prepared using a multistep synthetic scheme in which the key step was the Koenigs-Knorr condensation of 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide and benzyl 2,3-anhydro-β-D-ribopyranoside. Both silver oxide and silver trifluoromethanesulfonate were used as promotors in this synthesis, but the silver oxide system produced the highest yield (51%) of benzyl 2,3-anhydro-4-O-(2',3',4'-tri-O-acetyl-β-D-xylopyranosyl)-β-D-ribopyranoside. Preparation of xylobiose involved opening the epoxide of benzyl 2,3-anhydro-4-O-(β-D-xylopyranosyl)-β-D-ribopyranoside with hydroxide ion. The product mixture consisted of disaccharides with xylo- and arabino-reducing end units in the ratio of nine to one, respectively. Similarly, synthesis of 2',3',4'-tri-O-methyl-xylobiose involved the use of benzyl oxide to open the epoxide of benzyl 2,3-anhydro-4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-ribopyranoside. The product mixture contained disaccharides with xylo- and arabino-reducing end units in the ratio of twenty-four to one, respectively. In contrast, when hydroxide ion was used to open the epoxide of benzyl 2,3-anhydro-4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-ribopyranoside disaccharides with xylo- and arabino-reducing end units were formed in equal amounts. Carbon-13 nuclear magnetic resonance spectrometry was used extensively to characterize intermediate and final products.

Xylobiose and 2',3',4'-tri-O-methyl-xylobiose were degraded in 0.1M or 2.5M sodium hydroxide or 0.1M sodium hydroxide containing 2.4M sodium acetate at 30°C in a nitrogen atmosphere. Kinetic data were obtained by quantitative gas-liquid chromatographic analyses of the two disaccharides as their per-O-acetylated alditol derivatives. The pseudo-first-order rate constants increased for both disaccharides as the alkali concentration or ionic strength increased. The rate constant for xylobiose increased from 4.8×10^{-5} sec^{-1} to 6.85×10^{-5} sec^{-1} as the alkali concentration increased from 0.1M to 2.5M sodium hydroxide; the rate constant for 2',3',4'-tri-O-methyl-xylobiose increased from 4.45×10^{-5} sec^{-1} to 6.47×10^{-5} sec^{-1}. When the ionic strength of the 0.1M sodium hydroxide solution was increased to 2.5M with sodium acetate, the rate constants for xylobiose and 2',3',4'-tri-O-methyl-xylobiose almost increased to the level of the 2.5M sodium hydroxide reaction, i.e., the rate constants for xylobiose and 2',3',4'-tri-O-methyl-xylobiose were 6.35×10^{-5} sec^{-1} and 5.3×10^{-5} sec^{-1}, respectively. Therefore, ionization of hydroxyl groups in the glycose unit of a disaccharide has no effect on the degradation rate.

A reactive intermediate, 4-deoxy-2,3-pentodiulose, was formed in the degradation of both xylobiose and 2',3',4'-tri-O-methyl-xylobiose in 0.1M sodium hydroxide. This intermediate was formed from the reducing end unit after the peeling reaction and subsequently degrades to a series of acidic products.

The degradation products from the xylobiose and 2',3',4'-tri-O-methyl-xylobiose were identified as their trimethylsilyl derivatives by gas-liquid chromatography-mass spectrometry. Of these products, the most diagnostic products were those attributed to the reducing end unit of the disaccharides. With both disaccharides the major reducing end unit products consisted of glycolic, 2-hydroxybutyric, 3-hydroxypropionic, and D-xyloisosaccharinic acids. More fragmentation

products were produced at low alkalinity than at high alkalinity for the two disaccharides and 2',3',4'-tri-O-methyl-xylobiose produce more fragmentation products than xylobiose at 0.1M sodium hydroxide.

INTRODUCTION

PERSPECTIVE

The majority of chemical pulp in the United States and Europe is produced by alkaline pulping processes (1,2). The intent of these processes is to solubilize the lignin in order to free the cellulosic material, but significant degradation of the cellulose and hemicellulose also occurs, thus reducing the pulp yield. The ever increasing cost of pulpwood has stimulated modification of existing processes to raise the yield of fiber (3-6). To increase the yield of usable pulp, either the yield of carbohydrates must be increased, or the lignin must be retained in a usable form. To increase the carbohydrates in wood pulp, we need a better understanding of the alkaline reaction mechanisms that are operative during pulping. The present study investigates one aspect of these reaction mechanisms through the alkaline degradation of two model compounds.

LITERATURE REVIEW

This review is intended to give the reader some background on oxygen-free alkaline degradation of reducing carbohydrates, including cellulose and cellulose model compounds, and provide a discussion of the present state of knowledge of the degradation mechanisms.

SODIUM HYDROXIDE REACTIONS WITH CELLULOSE

The two main reactions that occur when cellulose is degraded in oxygen-free alkali are glycosidic bond cleavage and the peeling reaction (1,7,8). Glycosidic bond cleavage is important because random cleavage of the cellulose polymer decreases the degree of polymerization and also forms new reducing end-groups capable of reacting via the peeling reaction. Glycosidic bond cleavage becomes important at temperatures in excess of 100°C.

The peeling reaction (1,7-9) (Fig. 1) is a complex series of reactions that have been postulated (8,9) to start with rearrangement of the reducing end-group from the aldose (I) to the ketose (II) form. Abstraction of H-3 from the ketose (II) would lead to elimination of the cellulose residue as an oxyanion from C-4 of the resultant enediolate III. Benzilic acid type rearrangement of the resultant α-dicarbonyl compound (IV) would yield D-glucoisosaccharinic acid (V). The eliminated cellulose residue would be capable of further peeling in the alkaline system. It has been estimated that approximately 65 glucose units are lost from the cellulose chain before the peeling reaction is stopped (10).

The reaction that ends the peeling process is called the stopping reaction (Fig. 2). For this reaction, it has been postulated (1,7-9) that abstraction of H-2 from the aldose (I) would lead to elimination of the C-3 hydroxyl group from the resultant enediolate VII. Benzilic acid type rearrangement of the resultant α-dicarbonyl (VIII) would produce D-glucometasaccharinic acid (IX) with the cellulose residue attached at C-4. The cellulose polymer, with the acidic end-group, would be stable to further peeling.

The peeling-stopping reaction mechanisms as typically presented in the literature are too simplistic (11-14). For example, the reaction of hydrocellulose with alkali (11) produced a series of soluble three, four, and five carbon acids in addition to D-glucoisosaccharinic acid which was the expected peeling product. Similarly, an analysis of the cellulose end-groups after an alkaline cook indicated that 2-C-methylglyceric acid end-groups were present in addition to the conventional stopping reaction product (D-glucometasaccharinic acid end-group) (14). Although logical mechanisms were postulated to account for these products, the results indicated that reactions other than the postulated peeling and stopping reactions were also occurring.

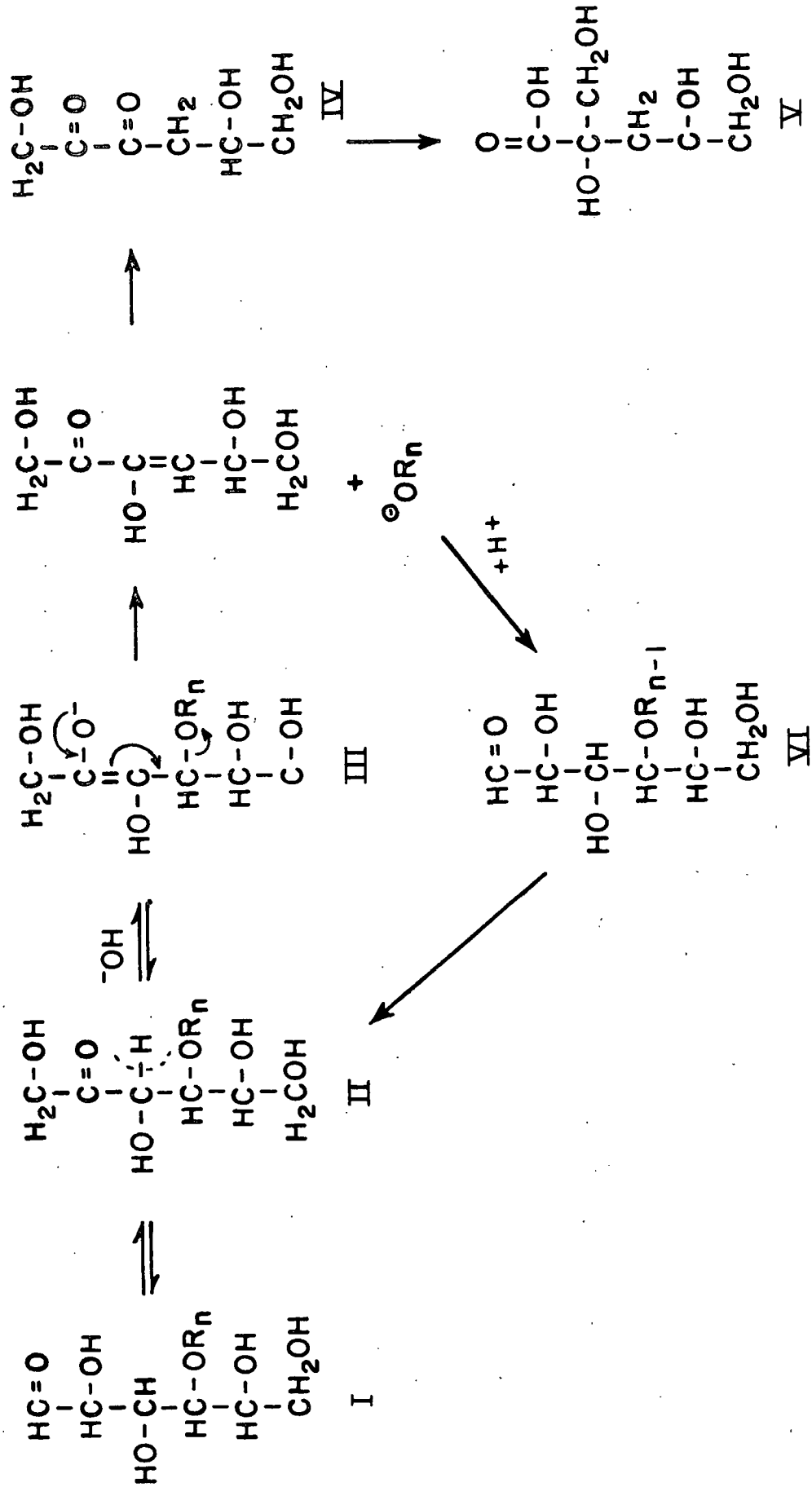


Figure 1. Postulated Mechanism for the Alkaline Peeling Reaction (1,7-9). R_n is the Remainder of the Cellulose Polymer

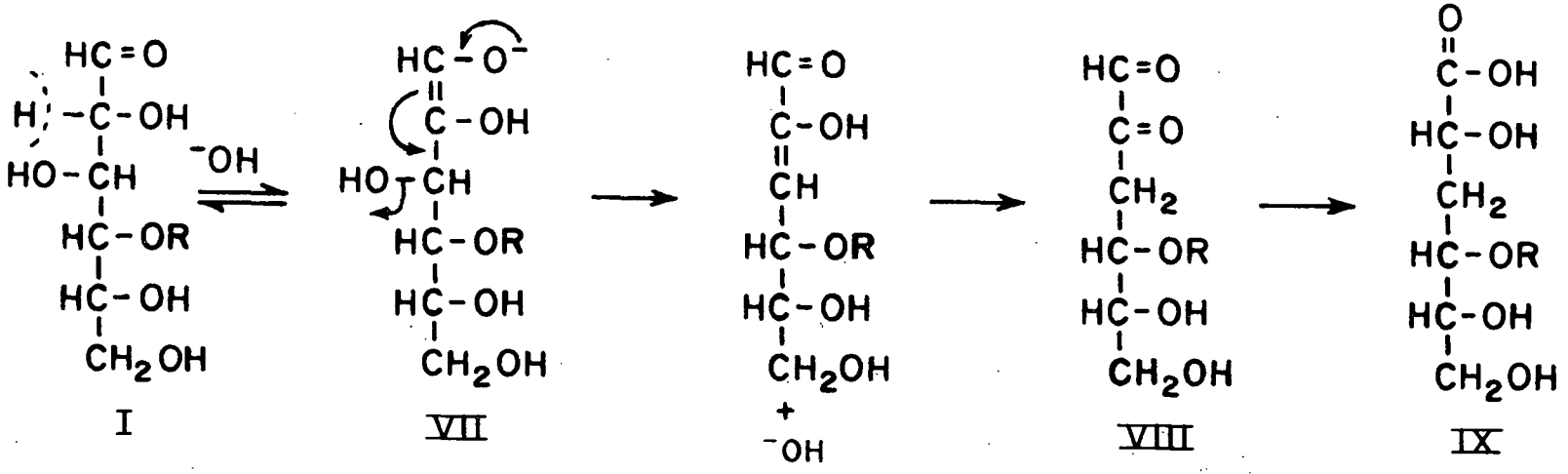


Figure 2. Postulated Mechanism for the Alkaline Stopping Reaction (1,7-9), where R is the Remainder of the Cellulose Polymer

MODEL COMPOUND STUDIES

Because of the complexity of the cellulose molecule, model compounds, which are more easily defined, are frequently used in the studies of alkaline reactions (15-40). Kenner, et al. (16,17,19-27,29,30) studied the reactions of numerous reducing sugars in dilute alkaline systems. They found (17) that 3-O-methyl-D-glucose degraded primarily to the D-glucometasaccharinic acid, but in a later study (35) small amounts of lactic and 3-deoxypentonic acids were also formed. Similarly, 4-O-methyl-D-glucose degraded primarily to D-glucoisosaccharinic acid (64%) and smaller amounts of other acidic products (36). Thus, substituents at C-3 or C-4 tend to be eliminated in preference to hydroxyl groups at other positions.

More recently it was shown (35,36) that D-glucometasaccharinic acid was a minor product of alkaline degradation of 4-O-methyl-D-glucose and 4-O-methyl-3,6-anhydro-D-glucose. Formation of the unsubstituted D-glucometasaccharinic acid indicates that elimination of both the C-3 and C-4 substituent must have occurred. A potential mechanism (35) for the reaction is shown in Fig. 3. Abstraction of H-2 from the aldose (X) would lead to elimination of OH-3 from the enediolate (XI). Subsequent elimination of the C-4 substituent from (XII) would provide the α,β-unsaturated dicarbonyl derivative (XIII). Addition of hydroxide ion at C-4 of intermediate (XIII) and subsequent benzilic acid type rearrangement of the resultant α-dicarbonyl species (XIV) would provide D-glucometasaccharinic acid (XV).

These studies (34-36) indicate that even with model compounds the peeling and stopping reactions are considerably more complex than typically envisioned.

EFFECT OF LEAVING GROUP ON THE DEGRADATION RATE

A study of alkaline degradation of 3-O-substituted-D-glucose derivatives has shown that (20,30) the rate of degradation depends on the substituent of C-3

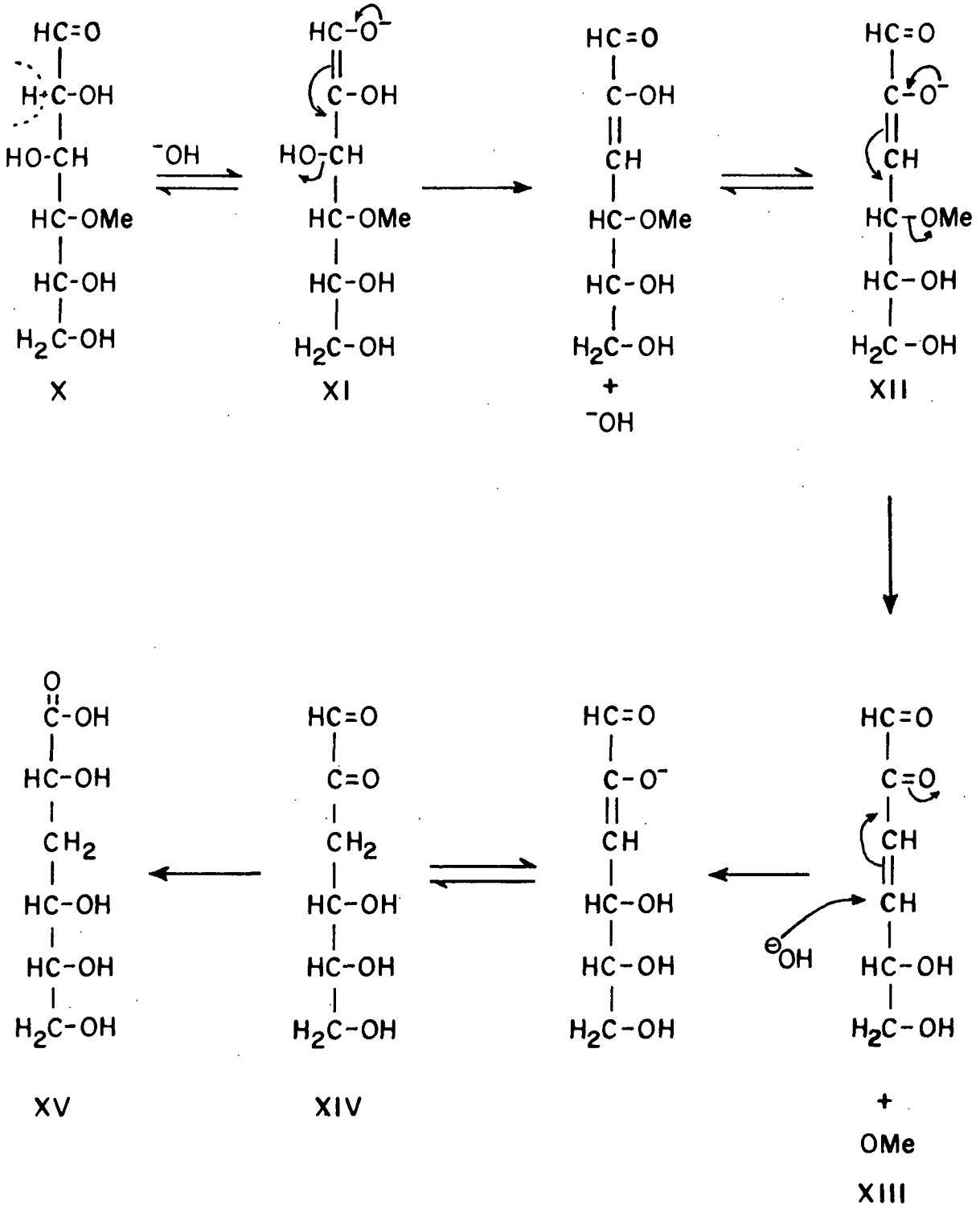


Figure 3. Potential Reaction Pathway for the Formation of D-glucometasaccharinic Acid from the Alkaline Degradation of 4-O-methyl-D-glucose (35)

(Table I). For example, 3-O-benzyl-D-glucose reacted twice as fast as the 3-O-methyl derivative. It was concluded (20,30) that the increase in the reaction rates was due to differences in leaving ability of the substituent and that the rate determining step in the reactions was probably elimination of the C-3 substituent.

TABLE I

EFFECT OF LEAVING GROUP ON REACTION RATE (20,30)[a]

3-O-Substituted-D-Glucose	Rate Constant, x 10^2 hr^{-1}
methyl	5.3
ethyl	3.0
n-propyl	2.9
iso-propyl	1.7
n-butyl	2.9
benzyl	10.1
β-D-glucopyranosyl	15.0

[a]The reducing sugars (0.0005-0.001M) were reacted in 0.04M lime water at 25°C in a nitrogen atmosphere.

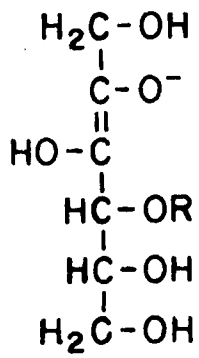
EFFECT OF BASE CONCENTRATION ON THE PEELING-STOPPING SEQUENCE

The potential effects of alkali concentration on reactions of reducing sugars has received little attention. Most studies of these reactions have been conducted at low alkali concentrations (0.01-0.1N) and the results have been extrapolated to more concentrated alkaline systems (2.5N). However, the distribution of products from a reaction in dilute alkali can be different from the product distribution obtained in more concentrated alkali.

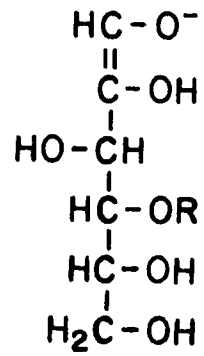
Sarkanen and Lai (12) studied the effect of alkali concentration on the degradation of amylose at 100°C (Fig. 4). The degradation of amylose was ca.

100% in 0.01-0.1N sodium hydroxide, while at concentrations of 0.1-5.0N sodium hydroxide the amount of degradation continually decreased to ca. 40%. From the amylose degradation data (Fig. 4), the peeling and stopping rate constants at each alkali level were calculated. The calculated stopping reaction rate constants increased from 0.42 hr^{-1} to 0.96 hr^{-1} as the alkali concentration increased 0.1N to 1.6N sodium hydroxide. In contrast, the calculated peeling rate constant increased from 286 hr^{-1} to 302 hr^{-1} with increases in alkali from 0.1N to 1.6N, respectively. The authors concluded (12) "... this kinetic pattern eliminates the direct participation of hydroxyl ions in the rate-determining step, and suggests the involvement of anionic species as reactive intermediates."

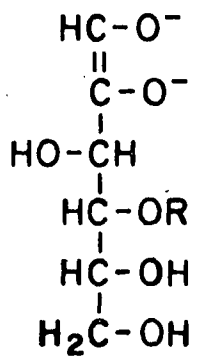
To explain these kinetic results, it was hypothesized (12,13) that at pH $\leq$ 13 the dominant reactive species was the enediol mono-anion XVI of the reducing end-group. The mono-anion XVI was postulated (12,13) to undergo only the peeling reaction which would lead to complete degradation of the polymer. However, there are two types of enediol mono-anions (XVI or XVII) possible in this system and inherent in Sarkanen and Lai's postulate (12,13) is the assumption that mono-anion XVII was not a contributing species since it could only form the stopping product.



XVI



XVII



XVIII

R is remainder of amylose polymer

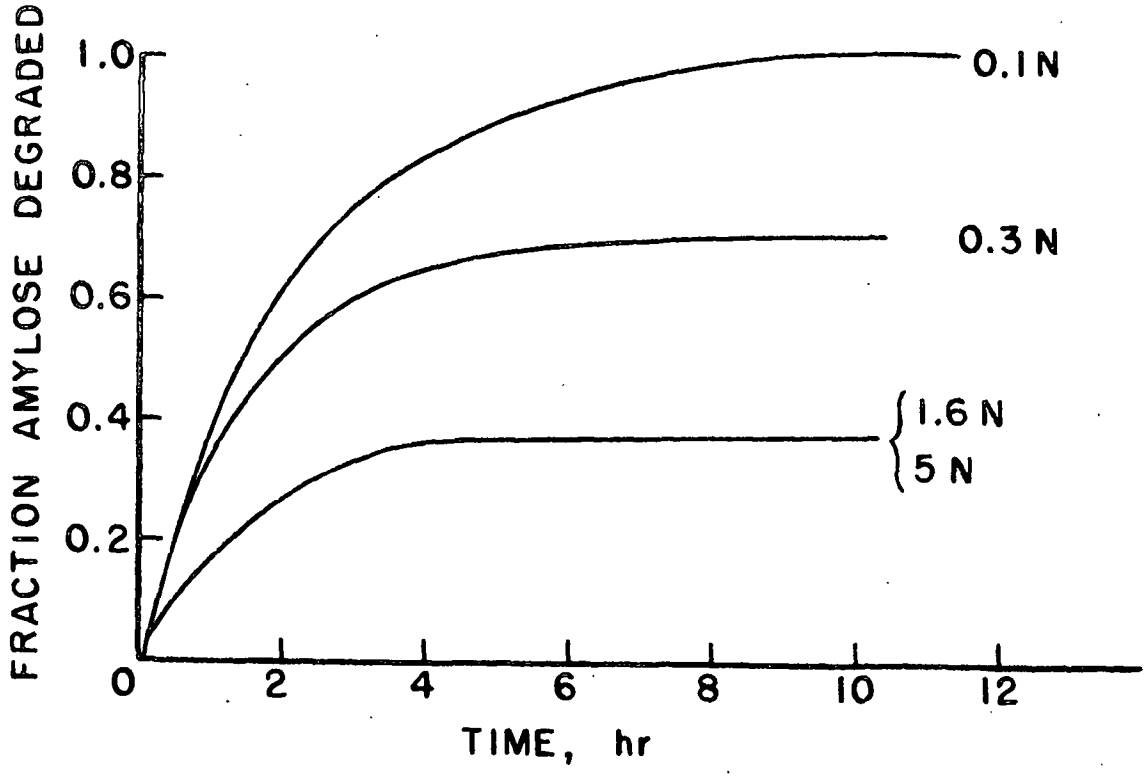


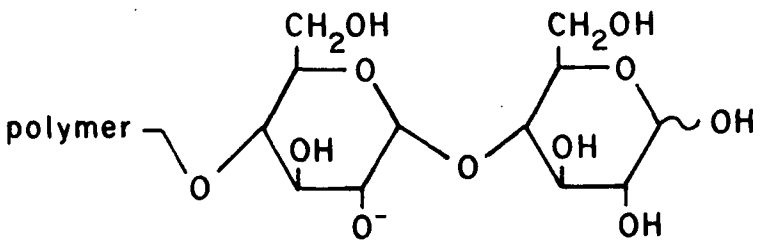
Figure 4. Effect of Alkali Concentration on the Degradation of Amylose (12)

To account for the decreased degradation at pH > 13 the authors (12,13) postulated that the enediol di-anion (XVIII) was becoming more important as a reaction intermediate. The di-anion (XVIII) was thought to undergo both the peeling and the stopping reactions (12,13). Therefore, they postulated that as the alkali concentration increased the di-anion (XVIII) concentration increased, resulting in more stopping product and decreased degradation of amylose (12,13).

In a similar study (41), xylotetraose was degraded at 60°C in alkali concentrations ranging from 0.0045N to 0.45N sodium hydroxide. While no detectable stopping product was formed at any reaction condition, increases in alkali concentration from 0.0045N to 0.45N did increase the peeling rate constant from 7.1 hr^{-1} to 25.7 hr^{-1}. These data indicated the effect of alkali concentration on the peeling rate constant was greater for xylotetraose than for amylose (12).

ALTERNATE EXPLANATION OF THE EFFECT OF ALKALI ON THE DEGRADATION OF AMYLOSE

The enediol di-anion concept (12,13) provides a potential explanation for the increased importance of alkali concentration in the degradation of amylose (Fig. 4). The mechanism (12) emphasized only ionization which occurs in the reducing end-group, but ionization of hydroxyl groups in the remainder of the polymer could also be important. For example, the viscosity of amylose reportedly increases as the alkali concentration is increased (42). This is attributed to ionization of hydroxyl groups throughout amylose which tends to extend the polymer chain. Although information pertaining to ionization of hydroxyl groups in polymers is limited, studies of monosaccharide glycosides (8) have shown that OH-2 and OH-6 are ionized quite easily, whereas, it is more difficult to ionize OH-3. It is logical to assume that hydroxyl groups in the glycose unit (XIX) attached to the reducing end-group of amylose would ionize to a greater extent as the alkali concentration increases.



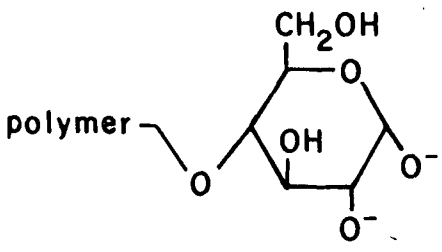
XIX

Based on the premise that ionization of hydroxyl groups, e.g., OH-2*, will occur in the glycose unit (XIX) attached to the reducing portion of amylose, an alternate explanation was developed to explain the dependence of amylose degradation on alkali concentration. The basic idea is that with increases in alkali concentration ionization of the C-2 hydroxyl groups in the glycose unit (XIX) should occur more easily than a second ionization occurring at OH-3 on the reducing end-group.

*Other hydroxyl groups could become ionized, but these are not as important to the following discussion.

Increased ionization of OH-3, which would decrease the amount of stopping reaction, would be more difficult due to the negatively charged enediol end-group. There are at least three potential explanations for the effect of the enediol on OH-3 ionization. First, the negative charge of the enediol anion may hinder abstraction of the OH-3 proton by the negatively charged hydroxide ion because of the repulsion of like charges. Secondly, the negatively charged enediol anion, which is an electron donating group, can also decrease the acidity of OH-3. Thirdly, hydrogen bonding between OH-3 and the enediol anion may occur and thus make it more difficult for the OH-3 proton to be abstracted by base. These arguments indicate that increased ionization of OH-3 of the reducing end unit would be relatively more difficult than increased ionization of OH-2 in the second glycose unit as the alkali concentration is raised.

The increased probability of OH-2 ionization in the second glycose unit (XIX) would make it more difficult for the polymer chain to be eliminated from the reducing end-group as a glycosyloxy anion (XX) because it would necessitate having two oxyanions on adjacent carbon atoms (43). The result would be that the probability of eliminating the glycosyloxy substituent would decrease with increasing alkali concentration relative to elimination of OH-3 from the reducing end-group. The result of the increased probability of C-3 hydroxyl group elimination would be formation of more stopping product at the higher alkali concentration. Thus, this type of explanation could also account for the decrease in degradation of amylose (12) at higher alkalinity.

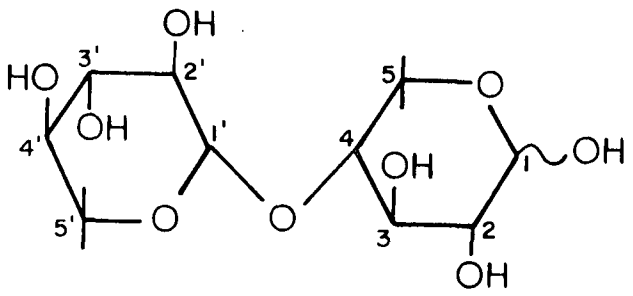


XX

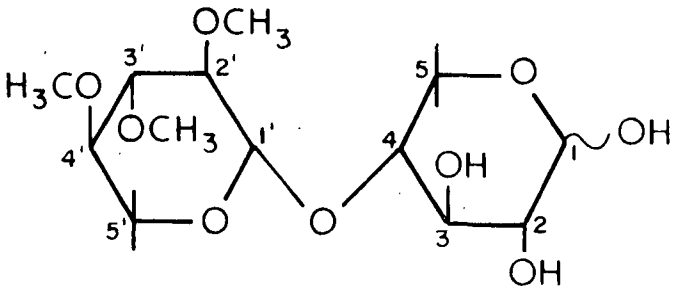
THESIS OBJECTIVE

The literature review has indicated that both the leaving group (21,24,26, 30) and alkali concentration (12,13,41,44,) affect the degradation of reducing carbohydrates, although the reasons for these effects are not necessarily clear. The objective of this thesis was to determine whether ionization of hydroxyl groups in the nonreducing glycose unit (leaving group) of a disaccharide affected alkaline degradation of the disaccharide.

The study involved degradation of two disaccharides, xylobiose (XXI) and 2',3',4'tri-O-methyl-xylobiose (XXII), at several alkali concentrations. These disaccharides are very similar except xylobiose (XXI) has free hydroxyl groups in the nonreducing glycose unit, whereas, in 2',3',4'-tri-O-methyl-xylobiose (XXII) these hydroxyl groups are etherified.* Therefore, effects of ionization of hydroxyl groups in the nonreducing glycose unit could be manifested in differences in degradation rates or products for the two compounds.



XXI



XXII

*Typically, the position of substituents in the nonreducing ring of a disaccharide are designated by a series of primed numbers, whereas, the reducing end unit has unprimed numbers. This nomenclature is used throughout this study.

RESULTS AND DISCUSSION

PREPARATION OF MODEL COMPOUNDS

SYNTHETIC SCHEME

The multistep synthetic scheme used to prepare xylobiose (XXI) and 2',3',4'-tri-O-methyl-xylobiose (XXII) is presented in Fig. 5. The first portion of the syntheses involved the use of specific blocking groups to obtain benzyl 2,3-anhydro-β-D-ribopyranoside (XXIII), which was obtained in 61% yield based on D-arabinose.

The next important synthetic step was condensation of the epoxide (XXIII) and 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide using either silver oxide or silver trifluoromethanesulfonate (triflate) as the promoter. The disaccharide product (XXIV) was prepared in 51% yield using the silver oxide reagent, but only 37% yield with silver triflate. The lower yield of (XXIV) in the silver triflate reaction was probably due to the sensitivity of the epoxide of XXIII and XXIV to the acidic reaction conditions (45).

The synthesis of xylobiose involved opening the epoxide of XXV with hydroxide ion and removal of the benzyl aglycon of the resultant benzyl β-xylobioside (XXVI) by catalytic hydrogenation. An earlier study (46) indicated that opening the epoxide of XXV with hydroxide ion resulted only in the xylo-configuration. However, this study has indicated that the reaction is not specific and that the epoxide of XXV is opened by hydroxide ion to form both the xylo- and arabino- configuration in the ratio of nine to one, respectively.

The presence of significant amounts of the contaminating arabino- isomer (∿10%) hindered efforts to characterize both xylobiose (XXI) and benzyl β-xylobioside (XXVI). However, acetylation of the crude products, followed by crystallization and deacetylation afforded chromatographically pure XXI and XXVI.

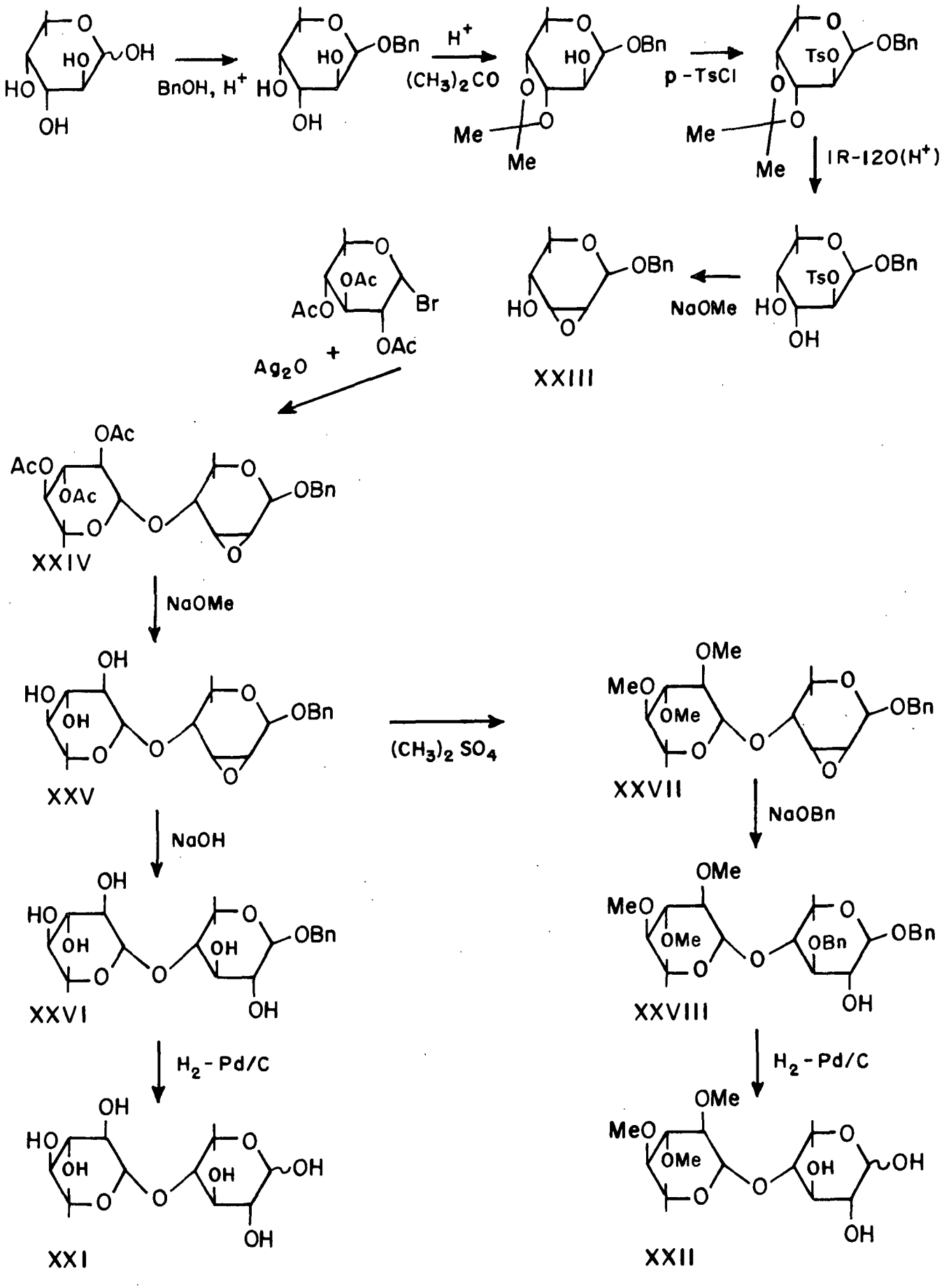
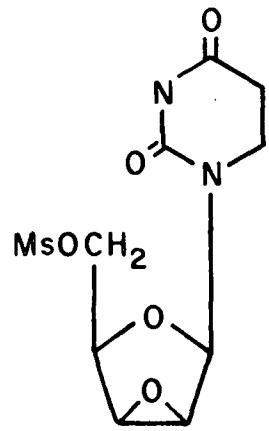


Figure 5. Synthetic Sequence for Preparation of Xylobiose (XXI) and 2',3',4'-tri-O-methyl-xylobiose (XXII)

The synthesis of 2',3',4'-tri-O-methyl-xylobiose (XXII) involved methylation of XXV to form XXVII and subsequent opening of the epoxide of XXVII by sodium benzyl oxide to form XXVIII. Removal of the benzyl groups of XXVIII by catalytic hydrogenation afforded chromatographically pure 2',3',4'-tri-O-methyl-xylobiose (XXII).

Initially, the epoxide of XXVII was opened using sodium hydroxide in aqueous dioxane. However, this resulted in approximately equal amounts of the isomers with the xylo- and arabino- configurations. Because the xylo- isomer was difficult to isolate from this mixture, a search for a nucleophile more selective than hydroxide ion was initiated.

Recently, sodium benzyl oxide in benzyl alcohol was used to open the epoxide of 1-(5'-O-methanesulfonyl-2',3'-epoxy-β-D-lyxosyl) uracil (XXIX) (47). The results indicated that nucleophilic attack occurred only at C-3, resulting in



XXIX

the isomer with the arabino- configuration. Similar selectivity occurred when the epoxide of XXVII was opened with sodium benzyl oxide since the isomers with the xylo- and arabino- configurations were obtained in a ratio of 24:1.

One plausible explanation for the increased stereoselectivity of benzyl oxide relative to hydroxide is that the bulky benzyl aglycon hinders attack at C-2 by the bulkier benzyl oxide nucleophile.

APPLICATION OF ^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROMETRY TO CARBOHYDRATE IDENTIFICATION

Carbon-13 nuclear magnetic resonance spectrometry (^{13}C-NMR) was used extensively throughout this work to confirm the structures of intermediate and final products. Representative ^{13}C-NMR spectra, those of β-xylobiose hexaacetate, xylobiose, and 2',3',4'-tri-O-methyl-xylobiose, are shown in Fig. 6-8, respectively. The ^{13}C-NMR spectrum of each compound synthesized and the structural assignments are presented in Appendix I.

A major advantage of ^{13}C-NMR is that each carbon atom frequently exhibits a distinct resonance. For example, the ten ring carbons of β-xylobiose hexaacetate (Fig. 6) each exhibit one distinct resonance (100.6-62.5 ppm). However, in some instances resonances can overlap and produce a signal more intense than would be expected for a single carbon atom. This is illustrated by the resonances of the acetyl methyl carbons (20.8-20.6ppm).

The spectra of reducing disaccharides, such as xylobiose (Fig. 7) and 2',3',4'-tri-O-methyl-xylobiose (Fig. 8) are more complex because of the equilibrium between the α and β anomers. However, the spectrum for an equilibrium mixture can be envisioned as the sum of the spectra of the α and β anomers. Frequently, the resonance frequencies of corresponding carbon atoms of the glycon moieties of α and β anomers of a reducing disaccharide are identical, resulting in these peaks being the most intense peaks in the spectrum. This is illustrated in both the xylobiose spectrum (Peaks 1, 6, 9, 12, and 13; Fig. 7) and the 2',3',4'-tri-O-methyl-xylobiose spectrum (Peaks 2, 5, 6, 7, and 14; Fig. 8). For xylobiose the β anomer predominates

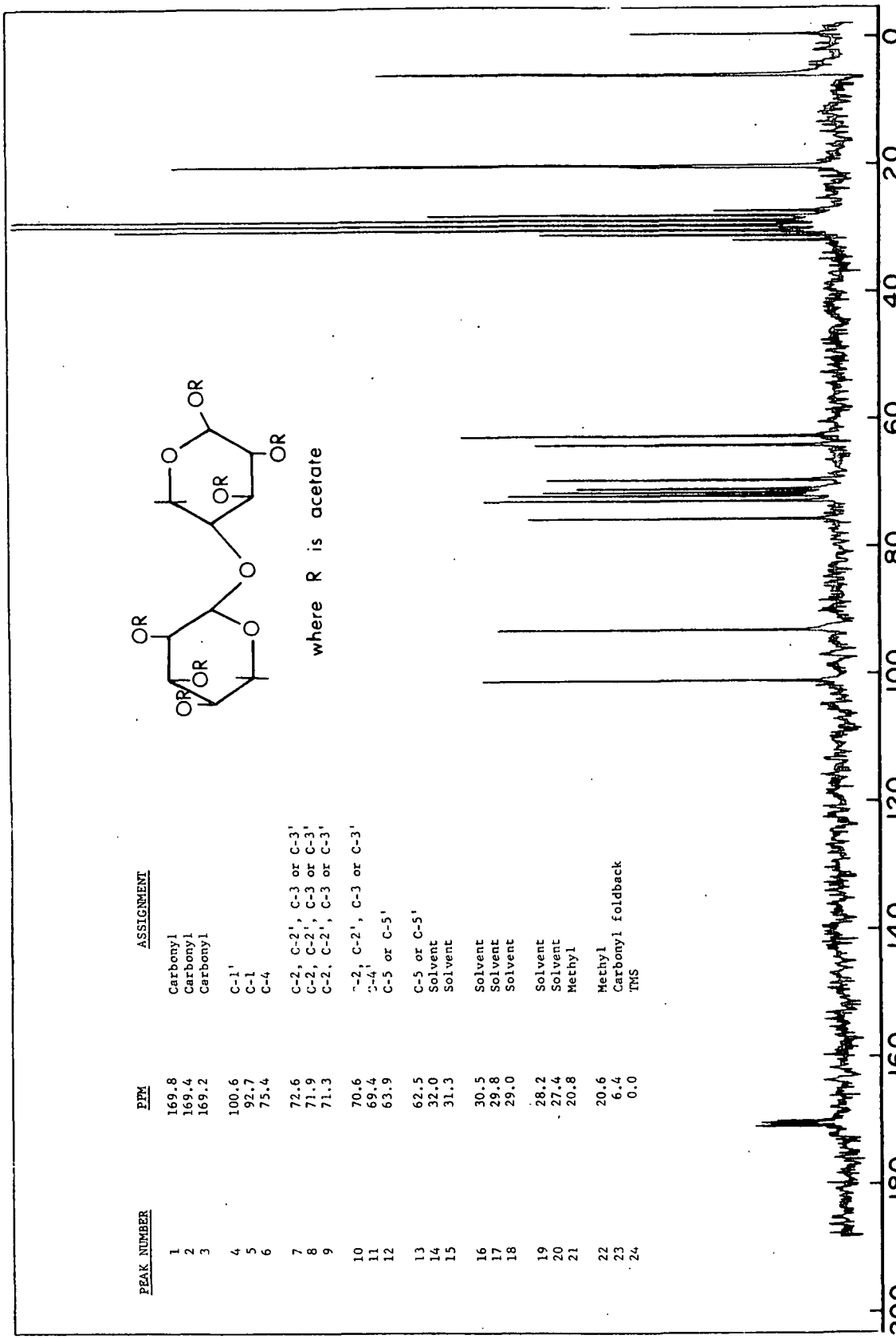


Figure 6. The ^{13}C-NMR Spectrum of β-Xylobiose Hexaacetate in Acetone -d_6

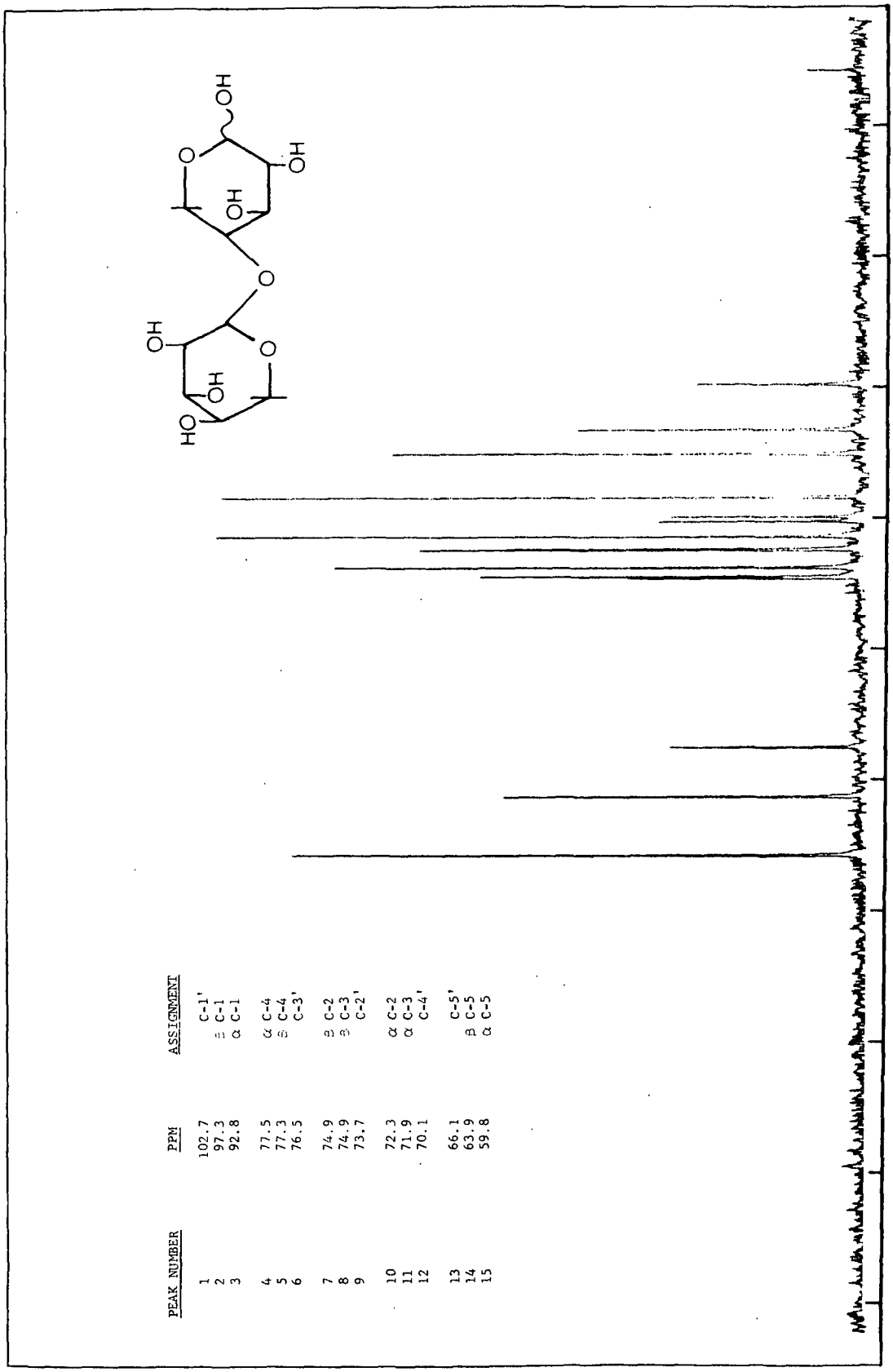


Figure 7. The ^{13}C-NMR Spectrum of Xylobiose in D_2O

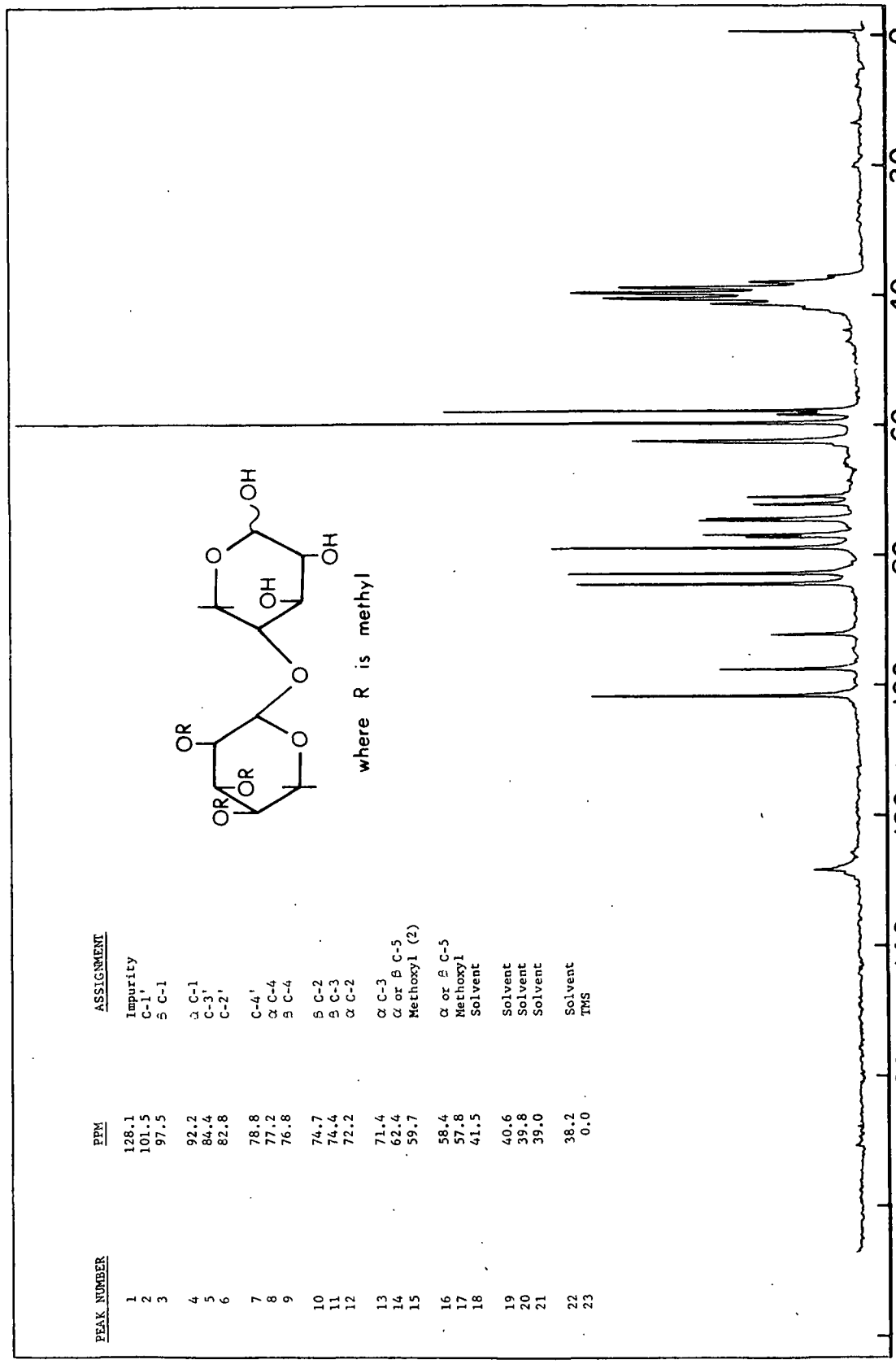


Figure 8. The ^{13}C-NMR Spectrum of 2',3',4'-Tri-O-Methyl-Xylobiose in Dimethyl Sulfoxide -d_6

in solution so the next most intense signals (Peaks 2, 5, 7, 8, and 14; Fig. 7) result from carbon atoms of the reducing end unit of the β anomer. Similarly, the least intense signals (Peaks 3, 4, 10, 11, and 15; Fig. 7) result from carbon atoms in the reducing end of the α anomer.

The utility of ^{13}C-NMR spectrometry in identifying reaction products can be illustrated for the reactions in which sodium hydroxide was used to open the epoxide of XXVII. If this reaction had been stereospecific there would only be two signals in the anomeric carbon region (105-95 ppm) of the ^{13}C-NMR spectrum of the disaccharide product. However, as shown in Fig. 9, there were four signals in this region of the spectrum. This information indicated that there were two disaccharide compounds present and that they were in almost equal concentrations. Two of these peaks (103.5 and 103.4 ppm) can be attributed to C-1' atoms and the other two peaks (101.5 and 98.1 ppm) attributed to C-1 atoms. Subsequent gas-liquid chromatographic (GLC) analyses verified that the reaction product contained equal amounts of disaccharides having the xylo- and arabino- configuration of the reducing end unit.

DEGRADATION STUDIES

GENERAL

A set of standard conditions was employed for alkaline degradation of the reducing disaccharides. The disaccharides (0.0012-0.0015M) were degraded at 30°C in a nitrogen atmosphere using 0.1M or 2.5M sodium hydroxide, or 0.1M sodium hydroxide containing 2.4M sodium acetate.

The disappearance of starting material was monitored by quantitative gas-liquid chromatography (GLC) of the per-O-acetylated alditol derivatives of the disaccharides.

PEAK NUMBER	PPM
1	136.9
2	136.7
3	128.2
4	127.8
5	127.6
6	103.5
7	103.4
8	101.5
9	98.1
10	84.7
11	84.8
12	82.9
13	79.3
14	78.8
15	78.2
16	76.9
17	75.6
18	73.3
19	72.4
20	70.6
21	70.4
22	69.6
23	69.0
24	63.3
25	62.4
26	61.6
27	60.5
28	58.7
29	0.0

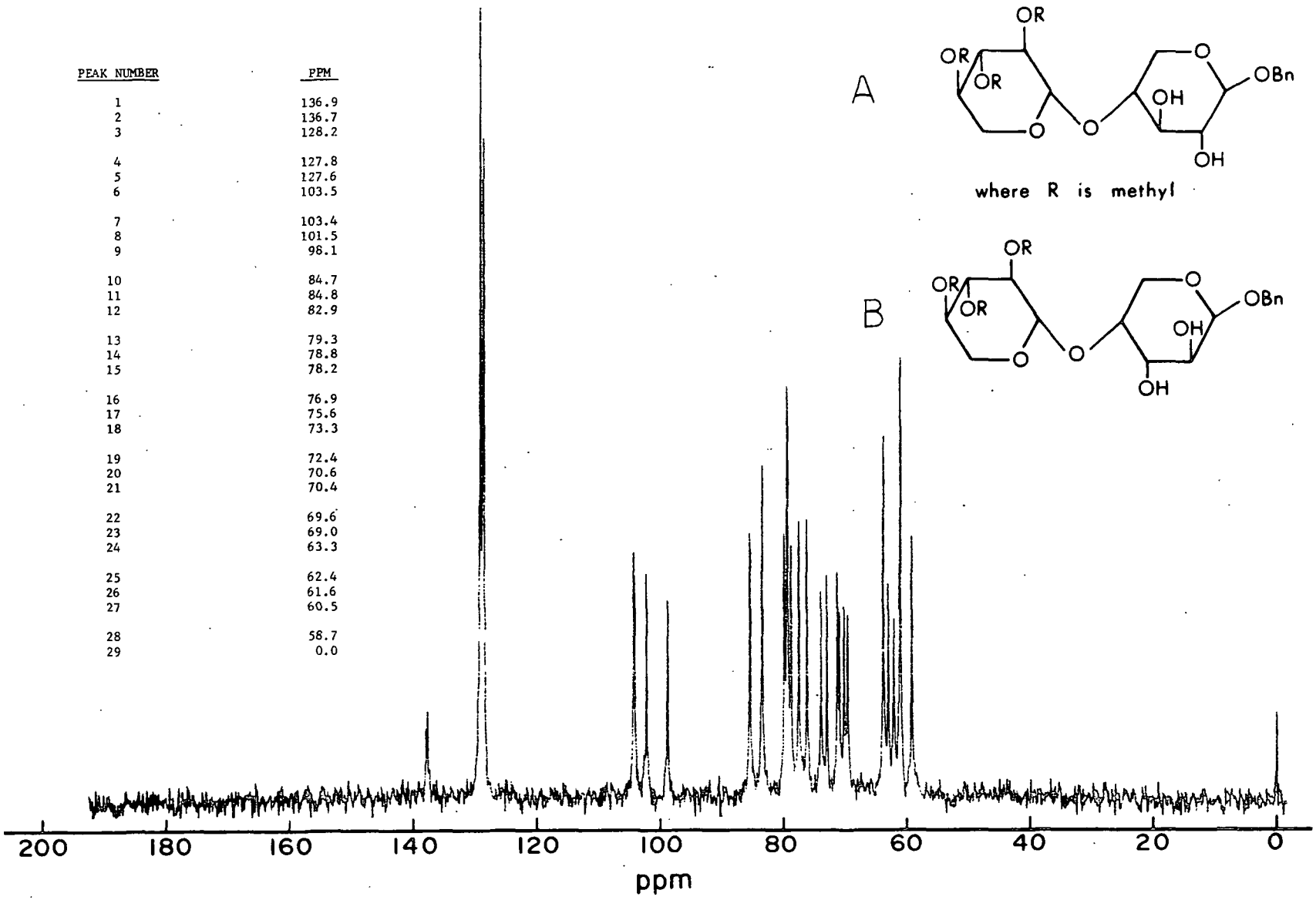


Figure 9. The ^{13}C-NMR Spectrum of the Product (A and B) From the Reaction of Benzyl 2,3-anhydro-4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-ribopyranoside and Sodium Hydroxide Obtained in Chloroform-d: A, Benzyl 4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-xylopyranoside; B, Benzyl 4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-arabinopyranoside

The carboxylic acid reaction end products were identified by gas-liquid chromatography-mass spectrometry (GLC-MS) analyses of the per-O-trimethylsilylated derivatives. The relative amounts of end products at each reaction condition were determined by semiquantitative GLC of the per-O-trimethylsilylated derivatives.

A reactive intermediate, 4-deoxy-2,3-pentodiulose, was identified by GLC-MS analysis of the reduced, per-O-acetylated derivative. A quantitative determination of this intermediate was performed by GLC analysis of the resultant alditol acetate.

KINETIC ANALYSIS

The disappearance of xylobiose and 2',3',4'-tri-O-methyl-xylobiose could be described by Eq. (1).

$$\frac{dC}{dt} = - k [C]^a \quad f \{[NaOH]\}, \tag{1}$$

where

k = specific rate constant, sec^{-1}

C = concentration of carbohydrate at time t, M

f {[NaOH]} = some function of the sodium hydroxide concentration

a = order with respect to carbohydrate

t = time

If the reaction is first order with respect to carbohydrate, as indicated in similar degradations of both cellobiose (48) and xylotetraose (41), and since the sodium hydroxide was present in large excess, its concentration remained essentially constant throughout the course of the reaction, integration of (1) provides (2).

$$\ln C/C_o = -k_r t, \quad (2)$$

where

C_o = concentration of carbohydrate at time zero, M

$k_r = k \cdot f\{[NaOH]\}$ = pseudo-first-order rate constant for the disappearance of carbohydrate, sec^{-1}

Since sodium acetate alone did not degrade either disaccharide Eq. (2) was also applicable for ionic strength reactions.

The degradation reactions were analyzed according to Eq. (2). Representative analyses for xylobiose and 2',3',4'-tri-O-methyl-xylobiose are shown in Fig. 10 and 11, respectively. The linearity of the data through 65% reaction is consistent with the assumption that, as inferred from the degradation of cellobiose (48) and xylotetraose (41), the reactions are first order in carbohydrate. Pseudo-first-order rate constants (k_r) for the degradations were determined by least squares analysis of the data according to Eq. (2).

The kinetic data of all analyses fit well with the straight line plot as indicated by correlation coefficients ranging from 0.995 to 0.998. The standard error of the rate constants was less than or equal to the experimental error of the GLC analysis method (i.e., $\pm$ 3.0%). Similarly, the reproducibility of duplicate experiments was $\pm$ 1.5%.

The degradation rate constants (k_r) are reported in Table II. For both disaccharides k_r increased as the alkali concentration was raised from 0.1M to 2.5M sodium hydroxide. Similarly, the rate constant also increased for both compounds when the alkali concentration was held at 0.1M and the ionic strength was raised to 2.5M with sodium acetate. Under comparable conditions xylobiose reacted faster than 2',3',4'-tri-O-methyl-xylobiose.

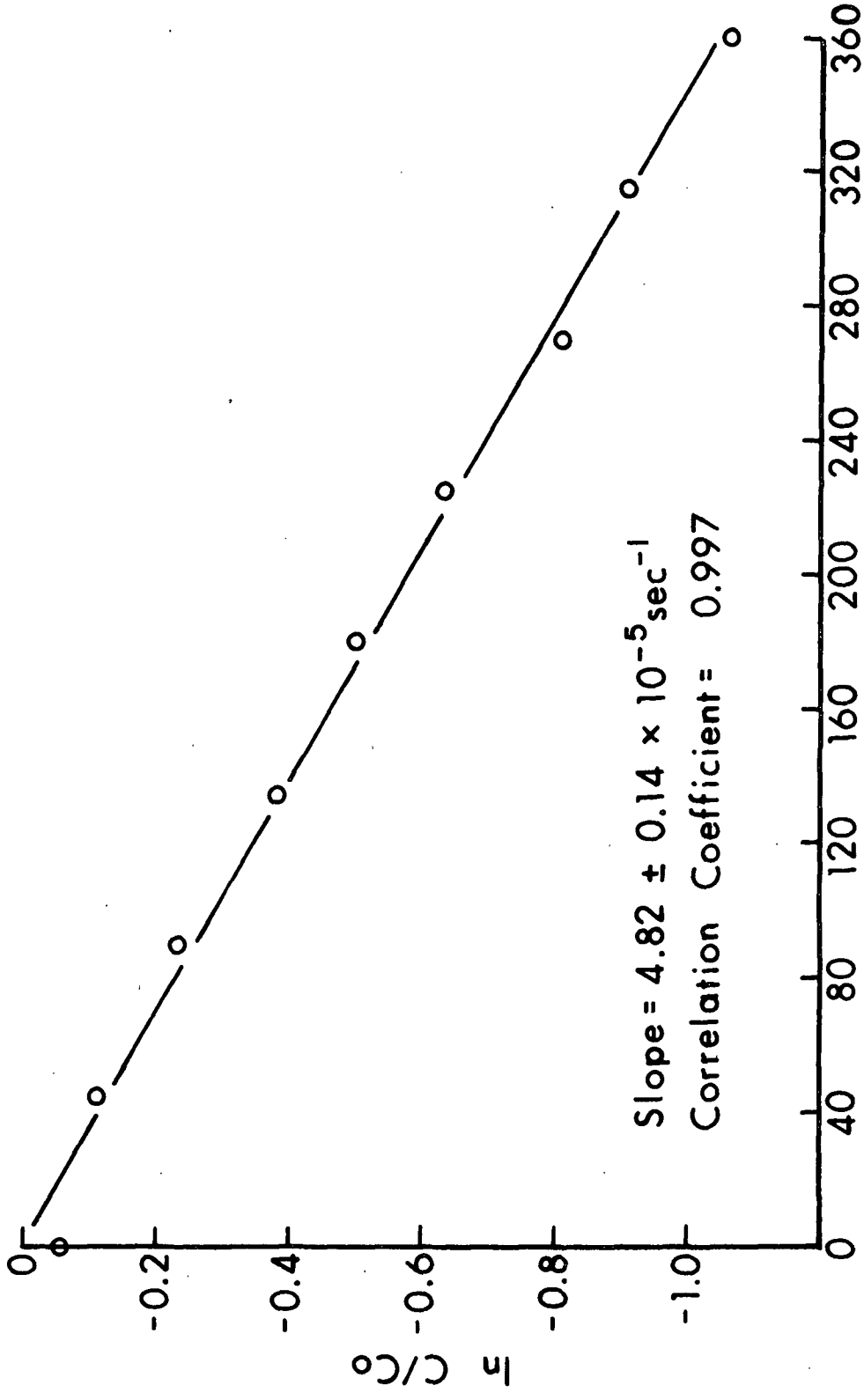


Figure 10. Degradation of Xylobiose (0.0015M) in 0.1M Sodium Hydroxide at 30°C

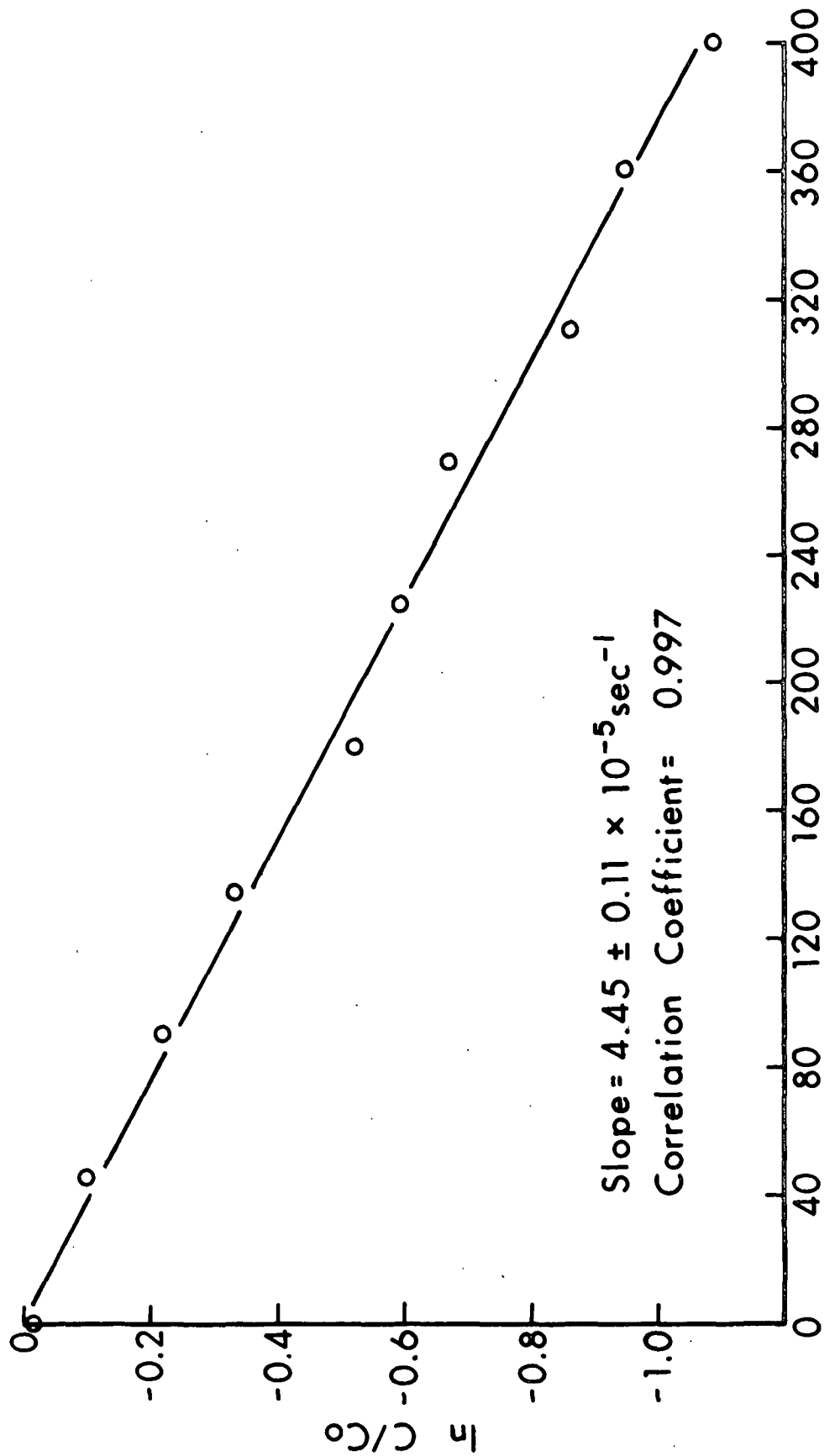


Figure 11. Degradation of 2',3',4'-tri-O-methyl Xylobiose (0.0012M) in 0.1M Sodium Hydroxide at 30°C

TABLE II

DEGRADATION RATE CONSTANTS DETERMINED AT 30°C[a]

Substrate	NaOH, M	NaOAc, M	$k_r \times 10^{-5}$ sec^{-1}[b]
Xylobiose	0.1	--	4.8 ± 0.14
Xylobiose	2.5	--	6.85 ± 0.16[c]
Xylobiose	0.1	2.4	6.35 ± 0.20
2',3',4'-tri-O-methyl-xylobiose	0.1	--	4.45 ± 0.11
2',3',4'-tri-O-methyl-xylobiose	2.5	--	6.47 ± 0.20[c]
2',3',4'-tri-O-methyl-xylobiose	0.1	2.4	5.3 ± 0.13

[a] Experimental data are reported in Appendix II.
[b] Pseudo-first-order rate constant determined using Eq. 2.
[c] Average value for duplicate experiments.

PRODUCT ANALYSIS

Reactive Intermediate

4-Deoxy-2,3-pentodiulose (XXX) was observed, as the alditol tetraacetate, in the kinetic chromatographic analyses of degradations of both disaccharides in 0.1M sodium hydroxide. Comparison of the mass spectra of the per-O-acetylated alditols obtained by reduction of the α-dicarbonyl (XXX) in sodium borohydride and sodium borodeuteride was the basis for identification of XXX, and is described in detail in Appendix III. Quantitative analysis of XXX, as its alditol acetate, showed that the α-dicarbonyl intermediate increased in concentration for 40-50% of the reaction and then decreased in concentration (Fig. 12). Interestingly, the concentration of the intermediate in the xylobiose degradation was approximately three times that of the 2',3',4'-tri-O-methyl-xylobiose reaction. The intermediate (XXX) was present in the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose, but not present in degradations of D-xylose or 2,3,4-tri-O-methyl-D-xylose which indicates that XXX is formed from the reducing end of

the two disaccharides. This α-dicarbonyl intermediate (XXX) is a postulated intermediate in the formation of D-xyloisosaccharinic acid (XXXI) and a series of fragmentation products (37).

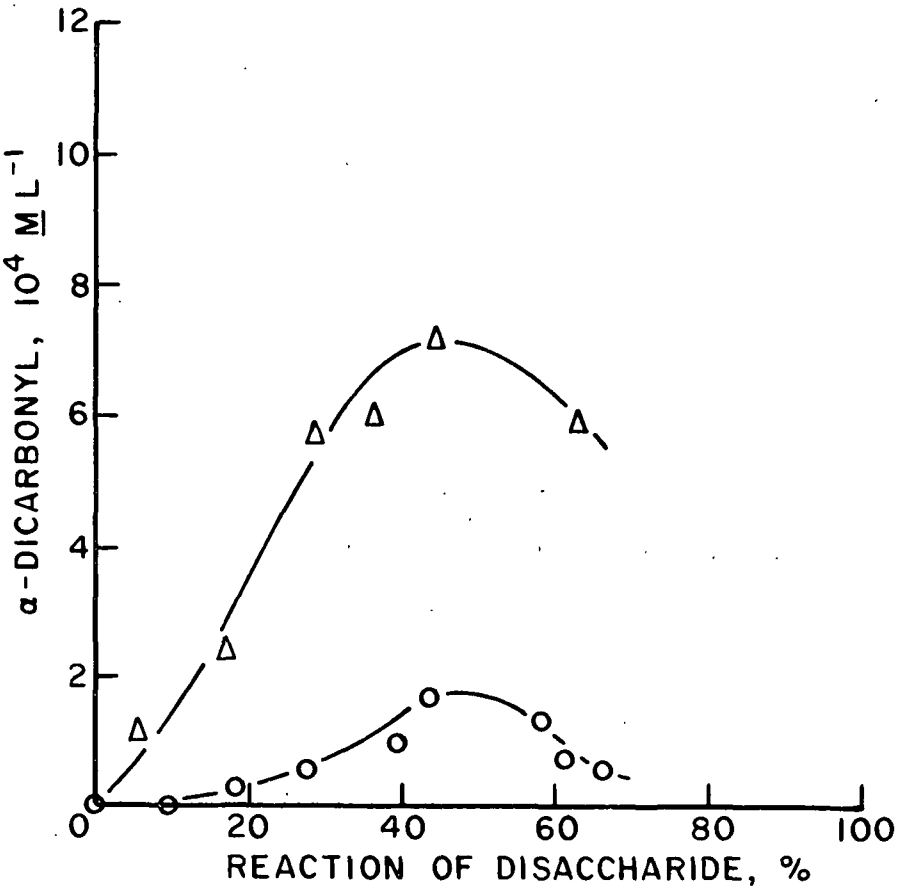
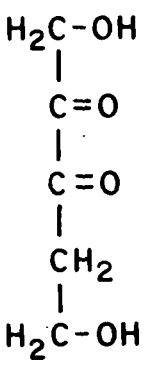


Figure 12. The Formation and Subsequent Degradation of 4-deoxy-2,3-pentodiulose (XXX) in Degradation of Xylobiose (△) and 2',3',4'-tri-O-methyl-xylobiose (○) in 0.1M Sodium Hydroxide at 30°C



XXX

Final Products

The final reaction products* from the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose were analyzed by GLC as their per-O-TMS derivatives at each set of reaction conditions. Representative chromatograms of the xylobiose and 2',3',4'-tri-O-methyl-xylobiose final products, and the product identities, are presented in Fig. 13 and 14, respectively. Mass spectral data used for product identification and their interpretation are reported in Appendix IV. The product analyses for xylobiose and 2',3',4'-tri-O-methyl-xylobiose are summarized in Table III and IV, respectively.

When xylobiose and 2',3',4'-tri-O-methyl-xylobiose were degraded, D-xylose and 2,3,4-tri-O-methyl-xylose, respectively, were shown to be formed as elimination products. The monosaccharides subsequently reacted to form a series of products. This reaction sequence is depicted for xylobiose and 2',3',4'-tri-O-methyl-xylobiose in Scheme 1. In order to determine which products originated from the eliminated monosaccharide, D-xylose and 2,3,4-tri-O-methyl-D-xylose were also degraded at each set of reaction conditions. Representative GLC chromatograms of D-xylose and 2,3,4-tri-O-methyl-D-xylose final products are presented in Fig. 15 and 16, respectively. The degradation products for D-xylose, shown in Scheme 1, consisted of lactic acid, 2,4-dihydroxybutyric acid, 3-deoxy-D-threo- and 3-deoxy-D-erythro-pentonic acids, and small amounts of glycolic acid. The products from the degradation of 2,3,4-tri-O-methyl-D-xylose, also presented in Scheme 1, consisted of a series of isomeric unknown products (9-12) and small amounts of two other unknown products (13 and 14). Table III and IV summarize the product analysis of xylose and 2,3,4-tri-O-methyl-xylose, and compare these products with the product analysis for xylobiose and 2',3',4'-tri-O-methyl-xylobiose, respectively.

*Products were determined from samples that were reacted in alkali for seven days. This corresponds to ca. 40 half-lives for the disaccharides in 0.1M sodium hydroxide.

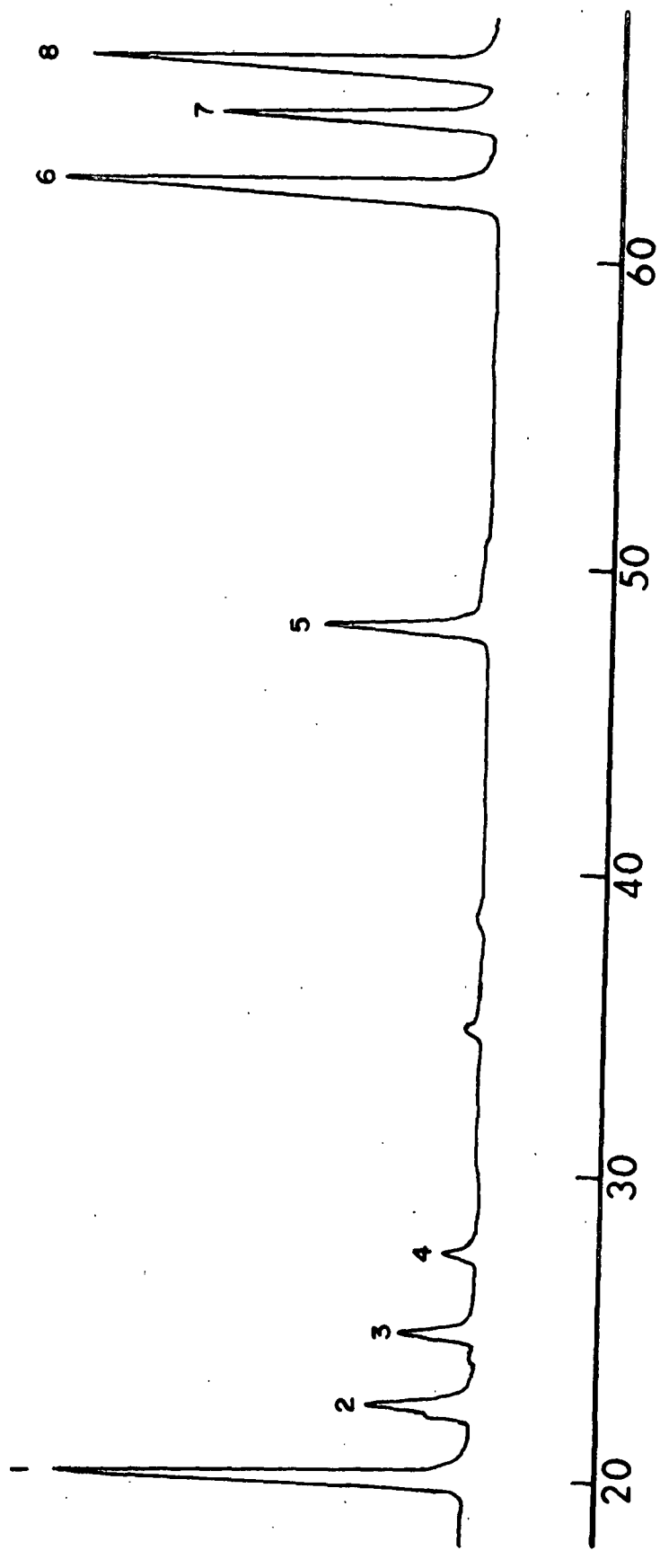


Figure 13. GLC Chromatogram of the Final Degradation Products (TMS Derivatives) of Xylobiose in 2.5M Sodium Hydroxide at 30°C: (1) Lactic Acid, (2) Glycolic Acid, (3) 2-Hydroxybutyric Acid, (4) 3-Hydroxypropionic Acid, (5) 2,4-Dihydroxybutyric Acid, (6) D-Xyloisosaccharinic Acid, (7) 3-Deoxy-D-threo-pentonic Acid, (8) 3-Deoxy-D-erythro-pentonic Acid

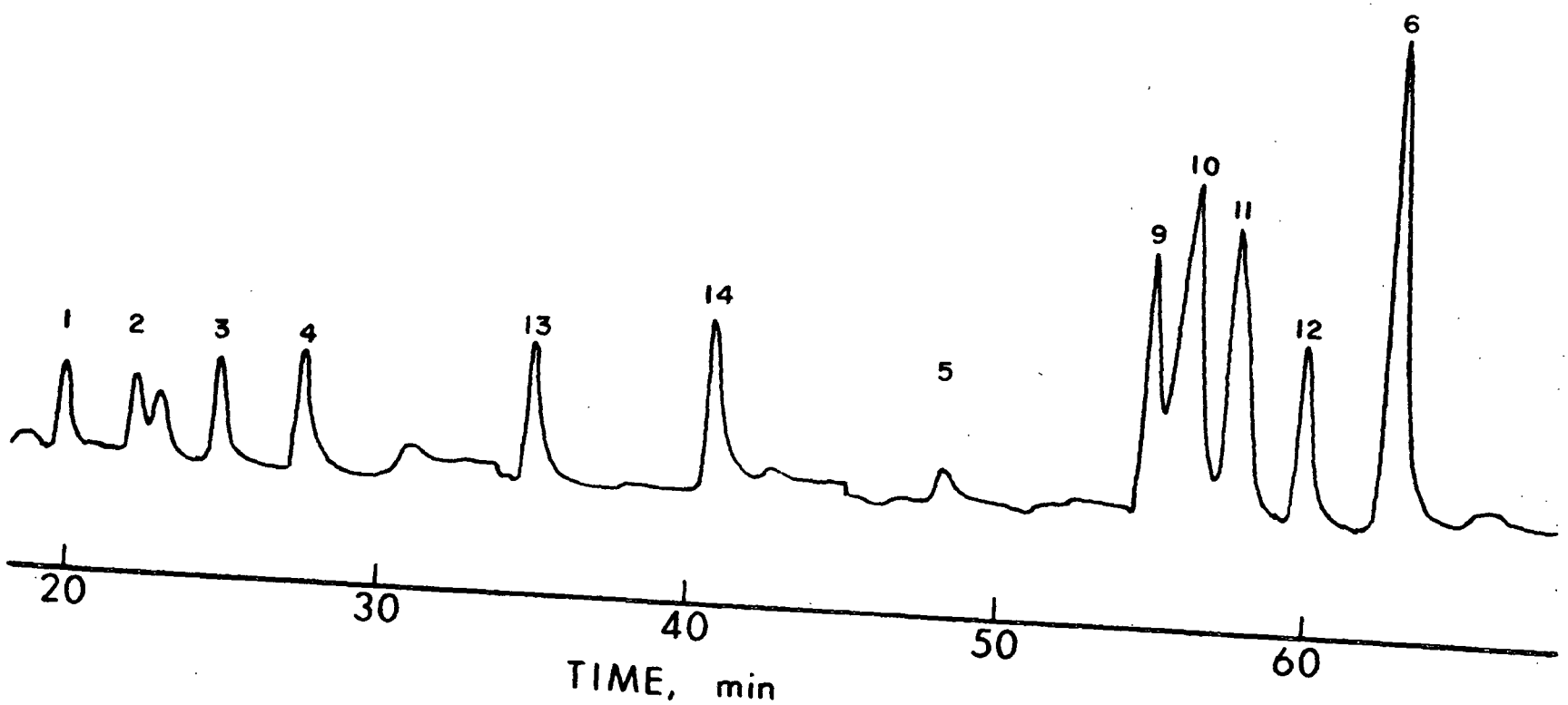
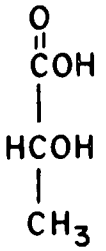


Figure 14. GLC Chromatogram of the Final Degradation Products (TMS Derivatives) of 2',3',4'-tri-O-methyl-xylobiose in 2.5M Sodium Hydroxide at 30°C: (1) Lactic Acid, (2) Glycolic Acid, (3) 2-Hydroxybutyric Acid, (4) 3-Hydroxypropionic Acid, (5) 2,4-Dihydroxybutyric Acid, (6) D-xyloisosaccharinic Acid, (9)-(12) Isomeric Unknowns, (13) Unknown, (14) Unknown

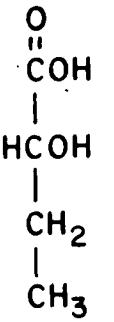


lactic acid

1

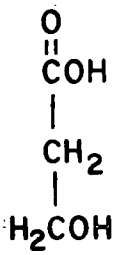
glycolic acid

2



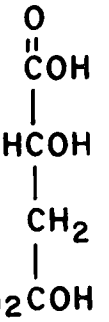
2-hydroxybutyric acid

3



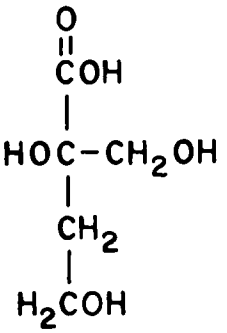
3-hydroxypropionic acid

4



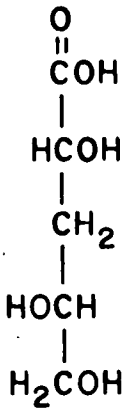
2,4-dihydroxybutyric acid

5



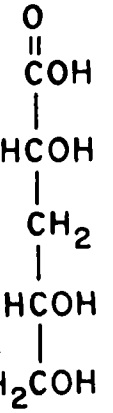
D-xyloisosaccharinic acid (XXXI)

6



3-deoxy-D-threo-pentonic acid

7



3-deoxy-D-erythro-pentonic acid

8

TABLE III

PRODUCTS FROM THE DEGRADATION OF XYLOBIOSE AND XYLOSE IN ALKALI[a]

Product	Acid Name	Xylobiose Product, %			Xylose Product, %		
		0.1M NaOH	2.5M NaOH	0.1M NaOH+ 2.4M NaOAc	0.1M NaOH	2.5M NaOH	0.1M NaOH+ 2.4M NaOAc
1	2-Hydroxypropionic acid (lactic acid[c])	20.7	19.5	20.7	49.8	40.8	43.5
2	Glycolic acid[c]	17.0	5.6	13.5	10.3	trace	8.9
3	2-Hydroxybutyric acid[b]	9.2	3.2	5.7	0.0	0.0	0.0
4	3-Hydroxypropionic acid[b]	8.7	1.2	5.5	0.0	0.0	0.0
5	2,4-Dihydroxybutyric acid[c]	3.6	7.5	2.1	6.4	15.6	7.4
6	3-Deoxy-2-C-(hydroxymethyl)-tetronic acid (D-xyloisosaccharinic acid)[b]	18.5	26.7	27.8	0.0	0.0	0.0
7	3-Deoxy-D-threo-pentonic acid	5.6	13.8	7.6	6.8	16.8	10.9
8	3-Deoxy-D-erythro-pentonic acid	16.7	22.4	17.2	24.8	25.8	29.7

[a] Quantities are the percent of observed products based on unit response factors.
[b] These are the products which definitely originate from the reducing end unit of xylobiose.
[c] A portion of these products originate from the reducing and nonreducing portion of xylobiose.

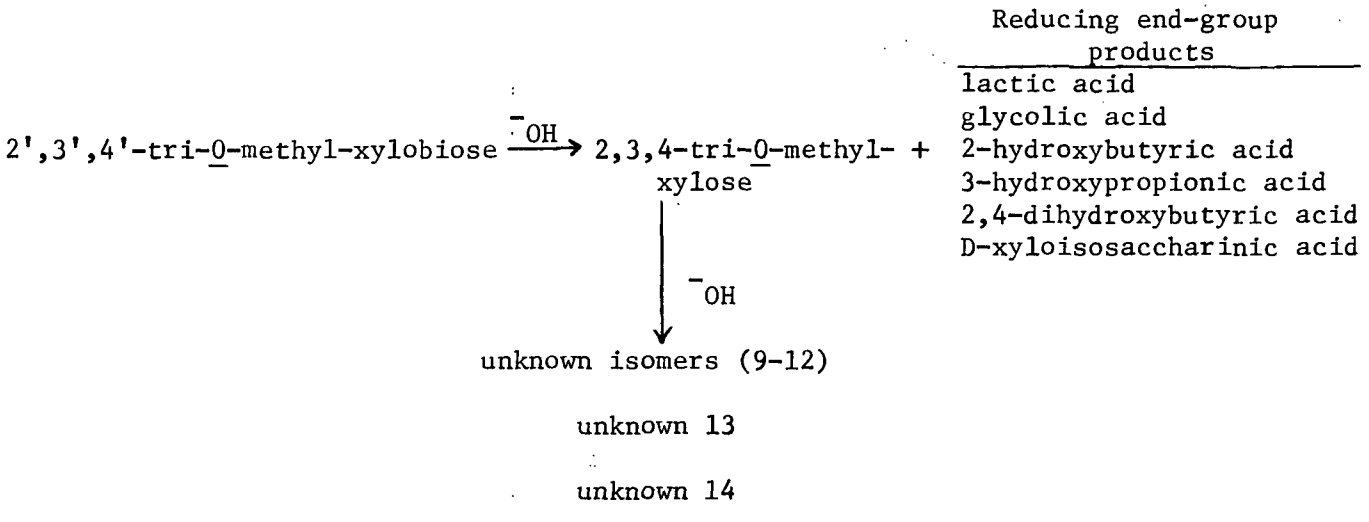
TABLE IV

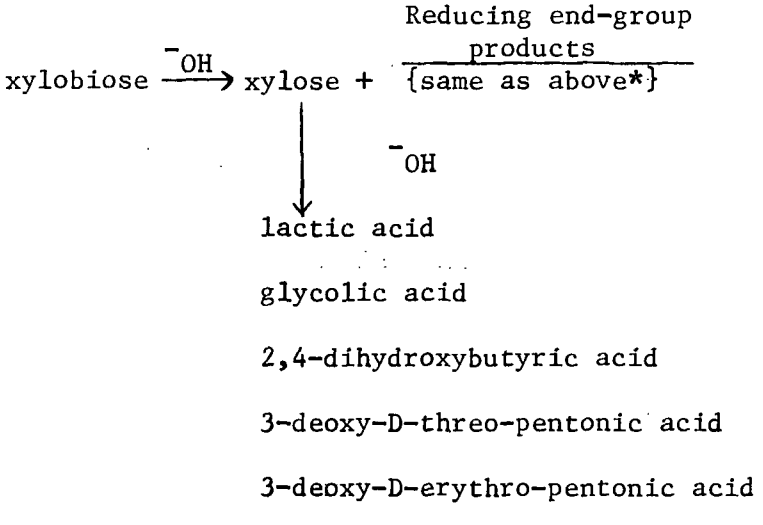
PRODUCTS FROM THE DEGRADATION OF 2',3',4'-TRI-O-METHYL-XYLOBIOSE AND 2,3,4-TRI-O-METHYL-XYLOSE IN ALKALI[a]

Product	Acid Name	2',3',4'-Tri-O-methyl-xylobiose Products, %			2,3,4-Tri-O-methyl-xylose Products, %		
		0.1M NaOH	2.5M NaOH	0.1M NaOH+ 2.4M NaOAc	0.1M NaOH	2.5M NaOH	0.1M NaOH+ 2.4M NaOAc
1	2-Hydroxypropionic acid (lactic acid)[b]	5.3	2.7	7.4	0.0	0.0	0.0
2	Glycolic acid[b]	14.1	3.9	10.9	0.0	0.0	0.0
3	2-Hydroxybutyric acid[b]	17.2	3.5	10.3	0.0	0.0	0.0
4	3-Hydroxypropionic acid[b]	16.2	4.9	13.8	0.0	0.0	0.0
13	Unknown	0.0	4.9	0.0	0.0	6.2	0.0
14	Unknown	3.8	6.8	6.9	9.2	9.1	17.2
5	2,4-Dihydroxybutyric acid[b]	3.2	0.0	3.0	0.0	0.0	0.0
9-12	Unknown (isomers)	31.9	55.0	35.6	90.8	85.2	82.8
6	3-Deoxy-2-C-(hydroxymethyl)-tetronic acid (D-xyloisosaccharinic acid)[b]	8.3	18.1	11.9	0.0	0.0	0.0

[a] Quantities are the percent of observed products based on unit response factors.
[b] These are the products originating from the reducing end unit of 2',3',4'-tri-O-methyl-xylobiose.

SCHEME 1





*Formation of lactic and 2,4-dihydroxybutyric acid from the reducing end-group of xylobiose are predicated on the results of degradation of 2',3',4'-tri-O-methyl-xylobiose.

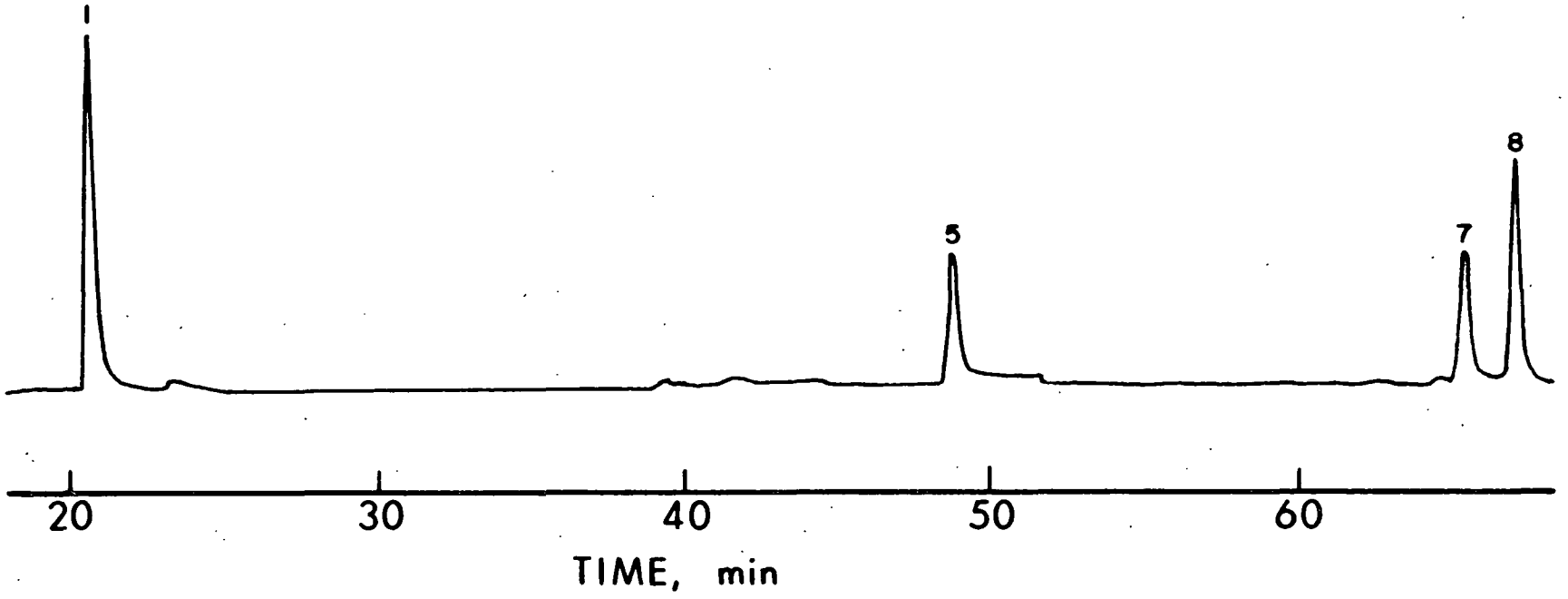


Figure 15. GLC Chromatogram of the Final Degradation Products (TMS Derivatives) of D-xylose in 2.5M Sodium Hydroxide at 30°C: (1) Lactic Acid, (5) 2,4-Dihydroxybutyric Acid, (7) 3-Deoxy-D-threo-pentonic Acid, (8) 3-Deoxy-D-erythro-pentonic Acid

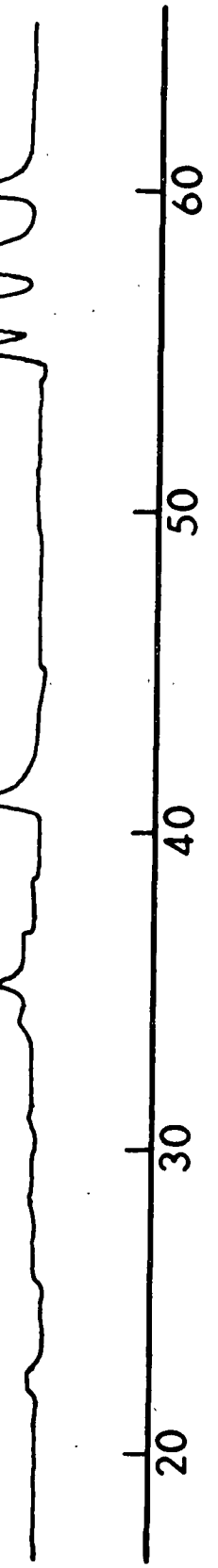


Figure 16. GLC Chromatogram of the Degradation Products (TMS Derivative) of 2,3,4-tri-O-methyl-D-xylose in 2.5M Sodium Hydroxide at 30°C: (9)-(12) Isomeric Unknowns, (13) Unknown, (14) Unknown

The reaction products (Table IV) from the degradation of 2',3',4'-tri-O-methyl-xylobiose contained several compounds (unknowns 9-12, 13, and 14) whose structures were not determined, although mass spectra of each product were obtained. The mass spectra of each of these unknowns were the same as the corresponding unknown from the degradation of 2,3,4-tri-O-methyl-xylose which indicates that these products all originate from the nonreducing end unit of 2',3',4'-tri-O-methyl-xylobiose. Therefore, the remainder of the products from the degradation of 2',3',4'-tri-O-methyl-xylobiose originate from the reducing portion of the disaccharide. These products consisted of D-xyloisosaccharinic acid (XXXI), glycolic acid, 2-hydroxybutyric acid, 3-hydroxypropionic acid, lactic acid and 2,4-dihydroxybutyric acid. In contrast, a direct comparison of the xylose and xylobiose final products was more complicated because some of the same products arise from the reducing and nonreducing portion of xylobiose. The products that definitely originate from the reducing end unit of xylobiose are D-xyloisosaccharinic acid, 2-hydroxybutyric acid, and 3-hydroxypropionic acid. A large percentage of glycolic acid was also formed from the reducing end unit of xylobiose although some was produced from xylose. In addition, lactic acid and 2,4-dihydroxybutyric acid were probably formed from the reducing end unit of xylobiose, but since these products were also formed from xylose the relative contribution of each could not be determined. The products attributed to the reducing end unit of the two disaccharides were thought to arise, at least partially, from subsequent reactions of 4-deoxy-2,3-pentodiulose discussed earlier.

In the 2.5M sodium hydroxide reactions the amount of reducing end unit products are considerably less than 50% of the total products. This is understandable in that the α-dicarbonyl (XXX) undergoes fragmentation reactions* producing volatile products (49), such as formic acid and formaldehyde, that were not analyzed in this

*Reactions that involve breaking carbon-carbon bonds of XXX that result in a series of lower molecular weight products.

system. The amount of volatile products was thought to increase to a certain extent as the alkali concentration increased (49).

EFFECT OF SODIUM HYDROXIDE

Reaction Rate

There is limited literature data pertaining to the effects of alkali concentration on the degradation of reducing saccharides. However, the applicable studies (12, 13, 30, 41) do indicate the degradation rate increases as the alkali concentration is increased. The literature indicates that there are basically two types of reaction rate-alkali concentration profiles as shown in Fig 17. In one type the rate tends to increase steadily with increases in alkali concentration (Fig. 17, curve A). In the second type (Fig. 17, curve B), the rate increases rapidly with small increases in alkalinity at low alkali concentrations. However, as the alkali concentration increases to higher levels the rate does not increase correspondingly, but tends to increase more slowly.

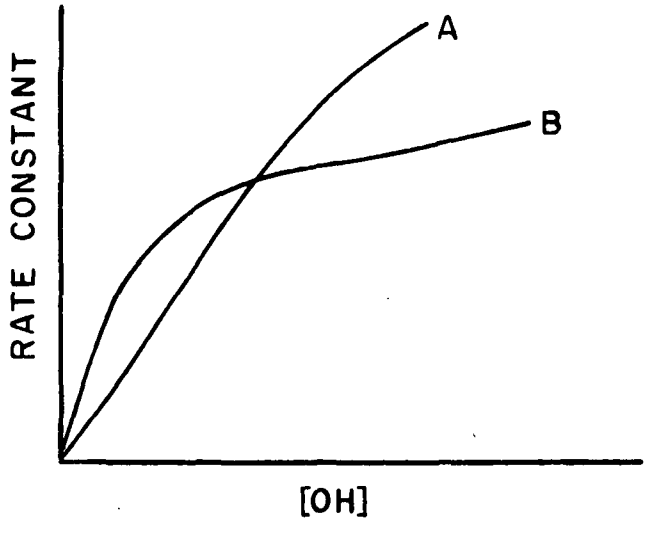


Figure 17. Effect of Alkali Concentration on the Pseudo Rate Constant for Degradation of Reducing Saccharides

The behavior depicted in Curve A is typical of certain monosaccharides. For example, the degradation rate constant for 3-O-methyl-D-glucose (30) increased steadily from 1.9×10^2 to 9.7×10^2 hr^{-1} as the alkalinity increased from 0.05N to 0.4N sodium hydroxide. In contrast, the degradation rates of amylose (12) and other saccharides (13,41) follow the pattern exhibited in Curve B. For example, the degradation rate constant for xylotetraose (41) increased from 14 to 21.3 hr^{-1} as the alkalinity was raised from 0.013N to 0.045N sodium hydroxide. However, a further increase in alkalinity from 0.045N to 0.45N only increased the rate constant from 21.3 to 25.7 hr^{-1}. Thus, in some cases increases in the degradation rate are not as great at high alkali concentration compared to low alkalinity.

In the present study the degradation rate constants for the two disaccharides did not increase substantially when the alkalinity increased from 0.1M to 2.5M sodium hydroxide. For example, the rate constant for xylobiose degradation increased from 4.6×10^{-5} to 6.8×10^{-5} sec^{-1} when the alkalinity increased from 0.1M to 2.5M sodium hydroxide; the rate constant for 2',3',4'-tri-O-methyl-xylobiose increased from 4.45×10^{-5} to 6.47×10^{-5} sec^{-1} with the same increases in alkalinity. Thus, the two disaccharides exhibit reaction rate-alkali concentration profiles similar to Curve B, in Fig. 17.

Degradation studies of certain polysaccharides (12,13) have indicated the rates of peeling and stopping are affected by alkali similar to Curve B, Fig. 17. In a study of amylose degradation, Lai and Sarkanen (12) indicated that the stopping reaction rate constant increased proportionately more than the peeling rate constant as the alkalinity increased, but both eventually reached a plateau at high alkalinity. However, since the inception of the present study, a new study of alkaline degradation of amylose (50) has indicated that the stopping and peeling rate constants are potentially influenced more by the concentration

of amylose than by the alkali concentration. Ziderman and Bel-Ayche (50) degraded a series of amylose solutions of varying concentration (0.01-0.5%) in a series of alkaline solutions (0.02-1.0N). The results, based on the amount of amylose degradation, are presented in Table V. The data indicate that complete degradation of amylose occurs at low substrate concentrations independent of alkalinity. However, less degradation occurs at all alkali concentrations when the amylose concentration is increased to 0.5%. The authors concluded that as the amylose concentration is increased at constant alkalinity, the peeling rate constant decreases and the stopping rate constant increases. They proposed that intermolecular association of the amylose polymer at high amylose concentration caused this effect. In light of this study, the peeling and stopping rate constants are potentially more affected by amylose concentration (50) than by alkali concentration as proposed earlier (12)*.

TABLE V

DEPENDENCE OF DEGRADATION ON AMYLOSE CONCENTRATION (50)

Sodium Hydroxide, M	Amylose Degraded After 20 hr at 98°C, %			
	0.01%[a]	0.04%	0.2%	0.5%
0.02	--	100	--	--
0.1	--	100	68	76
0.3	100	--	88	76
0.5	100	100	90	80
1.0	100	78	80	54

[a]Initial amylose concentration.

Reducing End-Group Products

The most diagnostic products from the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose are those which originate from the reducing end-group because that is where the peeling reaction starts. The products from the reducing end-group of 2',3',4'-tri-O-methyl-xylobiose included lactic acid, glycolic acid,

*Sarkanen and Lai (12) did not maintain a constant amylose concentration as they increased alkalinity, but increased amylose concentration as they increased alkalinity. This factor may have manifested their kinetic results presented in Fig. 4, in the Introduction.

2-hydroxybutyric acid, 3-hydroxypropionic acid, 2,4-dihydroxybutyric acid, and D-xyloisosaccharinic acid. For xylobiose, the distinction between reducing and non-reducing end-group products was more difficult to make because some of the same products were produced from both glycose units. For example, in 0.1M sodium hydroxide and 0.1M sodium hydroxide containing 2.4M sodium acetate small amounts of glycolic acid were produced from xylose degradation, yet glycolic acid was a major product from xylobiose degradation. This indicates that a large proportion of glycolic acid (perhaps 80% of the total) originated from the reducing portion of xylobiose. By analogy with the degradation products of 2',3',4'-tri-O-methyl-xylobiose, small amounts of lactic acid and 2,4-dihydroxybutyric acid were probably also formed from the reducing end-group of xylobiose, but because they were also dominant products from xylose degradation, the reducing end-group contribution could not be determined. The analyses did show that D-xyloisosaccharinic acid, 2-hydroxybutyric acid, and 3-hydroxypropionic acid all definitely originate from the reducing end-group of xylobiose.

Because it was difficult to determine the exact amount of some reducing end-group products for xylobiose, a direct comparison of all reducing end-group products between the two disaccharide systems cannot be made. However, for xylobiose, 2-hydroxybutyric acid, 3-hydroxypropionic acid, and D-xyloisosaccharinic acid were produced exclusively from the reducing end-group, therefore, a comparison of these three acids can be made between the two disaccharide systems. A summary of the reducing end-group products is given in Table VI. The amount of each acid present is relative to D-xyloisosaccharinic acid at each reaction condition, so a basis for comparison of these products between the two disaccharides is established.

The data in Table VI indicate that at 0.1M sodium hydroxide xylobiose produced half as much of either 2-hydroxybutyric acid or 3-hydroxypropionic acid as

TABLE VI

RELATIVE AMOUNTS OF REDUCING END PRODUCTS[a]

	Xylobiose			2',3',4'-tri-O-methyl-xylobiose		
	0.1M NaOH	2.5M NaOH	0.1M NaOH+ 2.4M NaOAc	0.1M NaOH	2.5M NaOH	0.1M NaOH+ 2.4M NaOAc
Lactic acid	0.0[c]	0.0[c]	0.0[c]	0.63	0.15	0.62
Glycolic acid	0.0[c]	0.21	0.0[c]	1.69	0.21	0.92
2-Hydroxybutyric acid	0.49	0.12	0.21	2.07	0.19	0.86
3-Hydroxypropionic acid	0.47	0.05	0.20	1.95	0.27	1.16
2,4-Dihydroxybutyric acid	0.0[c]	0.0[c]	0.0[c]	0.38	--	0.25
D-Xyloisosaccharinic acid	1.0	1.0	1.0	1.0	1.0	1.0

[a] These numbers are based on the data presented in Table III and IV. All amounts of acid products are relative to D-xyloisosaccharinic acid.
[b] Glycolic acid was not present in xylose degradation under these conditions, therefore, all glycolic acid originates from the reducing end-group.
[c] The amount of these products originating from the reducing end-group could not be determined.

D-xyloisosaccharinic acid. In contrast, at 0.1M sodium hydroxide 2',3',4'-tri-O-methyl-xylobiose produced approximately twice as much of either 2-hydroxybutyric acid or 3-hydroxypropionic acid as D-xyloisosaccharinic acid. At 2.5M sodium hydroxide the relative amount of fragmentation products compared to D-xyloisosaccharinic acid was drastically reduced and quite similar for both disaccharides. The data also indicates that the relative amount of fragmentation products decreased with respect to D-xyloisosaccharinic acid as the ionic strength of the 0.1M sodium hydroxide was increased to 2.5M with sodium acetate.

The fragmentation products and D-xyloisosaccharinic acid have typically been thought to arise from further reaction of 4-deoxy-2,3-pentodiulose (XXX) mentioned earlier. Reactions postulated to account for some of the products are shown in Fig. 18 (49). If the two disaccharides are reacting similarly they should produce similar amounts of fragmentation products relative to D-xyloisosaccharinic acid. In 0.1M sodium hydroxide xylobiose produced approximately twice as much D-xyloisosaccharinic acid as fragmentation products. In contrast, 2',3',4'-tri-O-methyl-xylobiose in 0.1M sodium hydroxide produced half as much D-xyloisosaccharinic acid as fragmentation products. A series of studies of the degradation of 4-deoxy-2,3-hexodiulose (37), 4-O-methyl-D-glucose (76), and cellobiose (77) showed that in dilute alkali the dominant product was D-glucoisosaccharinic acid with smaller amounts of fragmentation products. This pattern is quite similar to the product distribution for xylobiose. This indicates that 2',3',4'-tri-O-methyl-xylobiose produced more fragmentation products than would be expected, which suggests that at least the product determining step of the reaction is different for the two disaccharides.

Xylobiose and 2',3',4'-tri-O-methyl-xylobiose produced the same α-dicarbonyl intermediate (XXX), and the same products from the reducing end-group, although in differing amounts. This indicates that the two disaccharides do not degrade by two totally different mechanisms. However, the increased amount of fragmentation

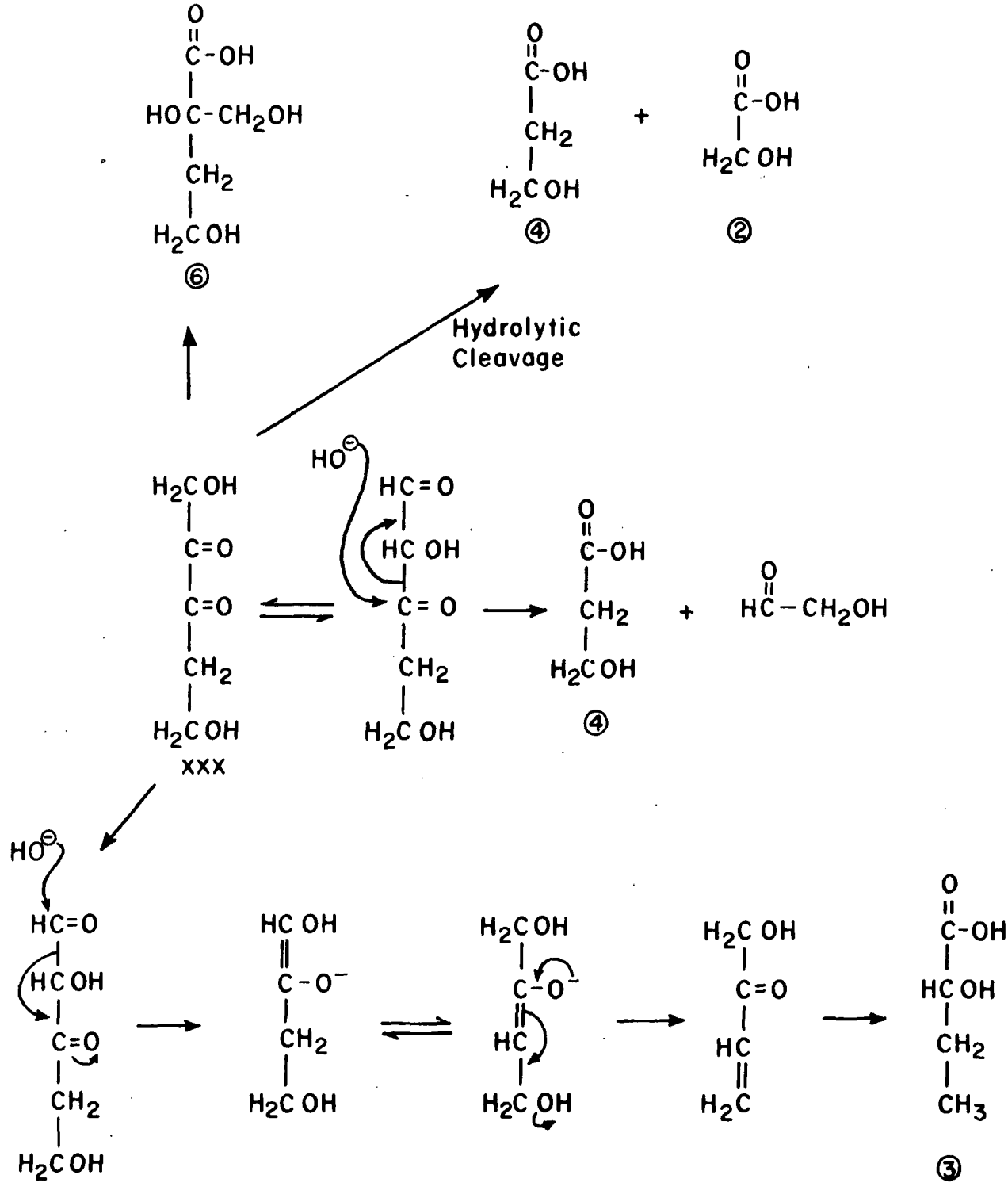


Figure 18. Postulated Mechanism for Formation of Glycolic (2), 3-Hydroxypropionic (4), 2-Hydroxybutyric (3), and D-Xyloisosaccharinic Acids (6), from the Degradation of 4-Deoxy-2,3-pentodiulose (XXX) in Alkali Based on Acidic Products from the Degradation of 4-Deoxy-2,3-hexodiulose in Alkali (49)

products and the decreased buildup of the α-dicarbonyl intermediate (XXX) for 2',3',4'-tri-O-methyl-xylobiose relative to xylobiose shows that the reaction pathways for the two disaccharides are not identical. It is known that some alkaline reactions of carbohydrates are altered by changing a substituent (30) or etherifying free hydroxyl groups (51,52), although the reason is not known. For example, 3-O-methyl-D-glucose degraded in alkali to D-glucometasaccharinic acid, whereas, 3-O-p-toluenesulfonyl-D-glucose degraded to 2-deoxy-D-ribose (30). The half-life of the 3-O-methyl derivative was 420 min, whereas, for the 3-O-p-toluenesulfonyl derivative the half-life was 3 min. This example illustrates a reaction pathway that is completely changed by the nature of a substituent, thereby exerting a drastic affect on both reaction rate and products. In contrast, comparison of the alkaline degradation reaction of 1,5-anhydro-cellobiitol (51) and 1,5-anhydro-2,3,6-tri-O-methyl-cellobiitol (52) indicated that more subtle changes in reaction pathway occurred due to the presence of the methyl substituents. In this reaction the methyl substituents of 1,5-anhydro-2,3,6-tri-O-methyl-cellobiitol did not change the reaction rate appreciably, but they did significantly alter the reaction products relative to cellobiitol. Thus, it is possible that the presence of the methoxyl groups in 2',3',4'-tri-O-methyl-xylobiose could facilitate another reaction pathway relative to xylobiose. These changes could occur anywhere in the overall reaction, but one possibility is that they occur prior to or during the rate-determining step of the reaction. In this case one reaction pathway could produce a product distribution similar to xylobiose, whereas the other pathway could produce predominantly fragmentation products. Although the identity of the alternate pathway is not known, the fact that the product distribution of the two disaccharides is similar in 2.5M sodium hydroxide indicates the alternate pathway must be less important at high alkalinity. In addition, if this type of reaction pathway is operative in the degradation of 2',3',4'-tri-O-methyl-xylobiose the similarity of

reaction rates of the two disaccharides at two extremely different reaction conditions would have to be fortuitous.

A second possibility could be that xylobiose and 2',3',4'-tri-O-methyl-xylobiose react along similar pathways up to and including the rate-determining step, and then differ in the product-determining step. This would result in different ratios of products from the reducing end-group of the two disaccharides. This would also be compatible with the decreased buildup of α-dicarbonyl intermediate (XXX) for 2',3',4'-tri-O-methyl-xylobiose relative to xylobiose if XXX is formed after the rate-determining step. In addition, the similarity of reaction rates for the two disaccharides at the two extremely different reaction conditions is also supportive of this type of argument.

The existence of a second reaction pathway prior to or during the rate-determining step of degradation of 2',3',4'-tri-O-methyl-xylobiose relative to xylobiose cannot be ruled out. But, if such a pathway is not operative and only a change in the product-determining step for the two disaccharide reactions account for the differing product distribution, the similarity of reaction rates for the two disaccharides at 0.1M and 2.5M sodium hydroxide indicates that ionization of hydroxyl groups in the nonreducing glycose unit has no effect on reaction rate. For hydroxyl group ionization to exert an effect, elimination of the C-4 substituent would have to occur prior to or during the rate-determing step of the peeling reaction. Thus, the absence of an effect would indicate that the rate-determining step of the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose occurs prior to elimination of the C-4 substituent. This is consistent with a recent study of the alkaline degradation of xylotetraose (41). It was concluded that the slow step of the reaction was formation of the tetrasaccharide with the ketose end-group. Elimination of the C-4 substituent was forty-five times faster than

the formation of the ketose end-group indicating that the elimination portion of the reaction was not rate-determining.

EFFECT OF INCREASED IONIC STRENGTH

As indicated earlier, studies (12,13,30,41) have shown that the degradation rate of reducing saccharides increases as the alkali concentration is raised. The effect has always been attributed to increases in the alkalinity, but the attendent increase in ionic strength could also affect the reaction rate. The effect of ionic strength has never been investigated in the alkaline degradation of reducing saccharides. Therefore, an attempt was made in this study to determine what effect increases in ionic strength have on the degradation rate of xylobiose and 2',3',4'-tri-O-methyl-xylobiose.

Initially, the ionic strength of the 0.1M sodium hydroxide solution was adjusted to 2.5M with sodium p-toluenesulfonate because the p-toluenesulfonate anion is a very poor nucleophile, but this proved experimentally impractical. This necessitated the use of sodium acetate in the ionic strength studies. Although the ion is somewhat more nucleophilic than the p-toluenesulfonate ion, it was not expected to degrade the disaccharides at these conditions (53).

Both disaccharides degraded more quickly in an alkaline solution with increased ionic strength. Specifically, the xylobiose rate constant increased from 4.8×10^{-5} to 6.3×10^{-5} sec^{-1} when the ionic strength of the 0.1M sodium hydroxide solution was increased from 0.1M to 2.5M by adding sodium acetate. Likewise, the rate constant for 2',3',4'-tri-O-methyl-xylobiose degradation increased from 4.45×10^{-5} to 5.4×10^{-5} sec^{-1} with a similar increase in ionic strength of the 0.1M sodium hydroxide solution. The rate constant for the xylobiose 2.5M ionic strength reaction was almost equal to the 2.5M sodium hydroxide reaction (6.8×10^{-5}

sec^{-1}), whereas, the 2',3',4'-tri-O-methyl-xylobiose rate constant for the ionic strength reaction was midway between the 0.1M and 2.5M (6.3×10^{-5} sec^{-1}) sodium hydroxide reactions. The following discussion examines potential explanations for the increased degradation rate in the ionic strength reactions.

As expected (53), neither disaccharide degraded in 2.5M sodium acetate.* This indicated that the sodium acetate did not directly cause the elimination of the C-4 substituent (i.e., cause additional peeling). However, the sodium acetate, in combination with sodium hydroxide, obviously caused an increase in the reaction rate via a salt effect.

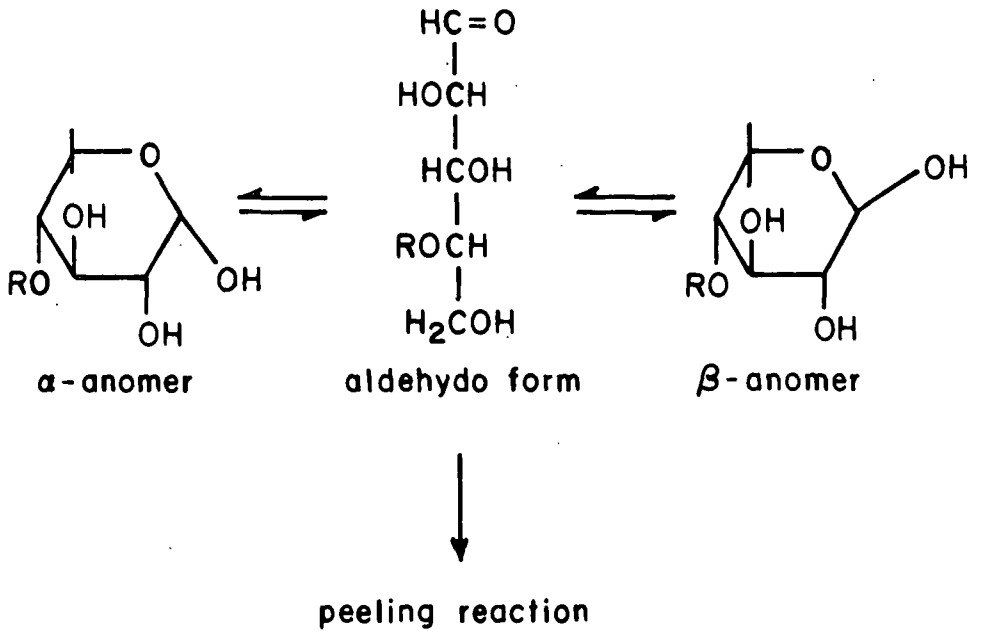
Salt effects are divided into two general categories, primary and secondary effects. A primary salt effect occurs because the salt increases the activity coefficient of one of the reactants that combine in the rate-determining step of the reaction. In this system the salt would have to increase the activity coefficient of sodium hydroxide, carbohydrate, or both. Limited data (54) suggest that increasing salt concentration actually decreases the activity coefficient of sodium hydroxide. The effect of the salt on the activity coefficient of the carbohydrate is not known.

In contrast, a secondary salt effect influences the equilibrium of certain fast, reversible reactions involving ions, thereby affecting the rate (55). The peeling reaction is a series of reversible reactions involving ionic intermediates, therefore the ionic strength of a solution has the potential to affect one or more of these reactions. Of these reactions, mutarotation of reducing sugars, which can be affected by changes in both base (56-58) and salt concentration (58,59), is the most plausible part of the peeling reaction to be influenced by ionic strength changes. Therefore, the following discussion examines how the mutarotation

*No reaction after three days. This corresponds to ca. 18 half-lives for the disaccharides in 0.1M sodium hydroxide.

reaction could increase the rate of degradation of the two disaccharides through additions of salts.

Mutarotation involves reactions of the reducing saccharide to form the α and β hemiacetal anomers, and the aldehydo isomer as shown below. The aldehydo intermediate is quite important because it is the initial intermediate in the postulated peeling reaction presented in Fig. 1. Although the concentration of this intermediate would be quite low (< 1%), its concentration is known to increase as the alkali concentration increases (56-58). Since the aldehydo isomer is a key reaction intermediate in the peeling reaction, an increase in its concentration would increase the peeling reaction rate.



where R is the remainder of the saccharide.

Salts, such as sodium acetate, are known to catalyze the mutarotation reaction (58,59,78), and their effect on the concentration of aldehydo intermediate is probably to increase it. The weakly acidic α and β anomers (hemiacetals) should be prone to ionize in the basic sodium acetate solution which should favor the reaction toward formation of the aldehydo intermediate. The reverse

reaction, which is already quite fast, should not increase as much. This type of behavior was observed for 2,3,4,6-tetra-O-methyl-D-glucose where the observed mutarotation rate constant (sum of the forward and reverse reaction) increased as the sodium acetate concentration increased (78). This indicates that the concentration of the aldehydo intermediate is probably increasing. In the present study, an increase in aldehydo concentration due to an increase in sodium acetate concentration would provide more aldehydo intermediate to react further with the alkali. Thus, this provides a reasonable explanation for the increased reaction rates in the ionic strength reactions.

CONCLUSIONS

Ionization of hydroxyl groups in the nonreducing glycose unit of a disaccharide has no effect on the reaction rate. The possibility that an additional reaction pathway, totally different from those for xylobiose, exists in the degradation of 2',3',4'-tri-O-methyl-xylobiose cannot be ruled out. However, the similarity of reaction rates for the two disaccharides at the different reaction conditions indicates that, most likely, only the product-determining steps are different. If this is true, the similarity of reaction rates for xylobiose and 2',3',4'-tri-O-methyl-xylobiose at two extreme reaction conditions indicates that elimination of the C-4 substituent occurs subsequent to the rate-determining step of the reactions. Otherwise, ionization of hydroxyl groups in the eliminated glycose unit should affect its leaving ability and hence, the rate of reaction.

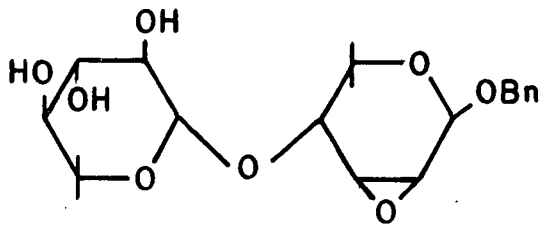
The increase in the degradation rate of the two disaccharides as the ionic strength increases is probably due to a shift in equilibrium toward formation of more of the aldehydo form of the reducing end-group.

4-Deoxy-2,3-pentodiulose (XXX) is a reactive intermediate in alkaline degradations of xylobiose and 2',3',4'-tri-O-methyl-xylobiose. It originates from the reducing end-group of the two disaccharides. This intermediate (XXX) was detected and identified in degradations in 0.1M sodium hydroxide. It was not detected in 2.5M sodium hydroxide reactions, probably because of its high reactivity at these conditions. In addition, XXX is more important in the degradation of xylobiose than 2',3',4'-tri-O-methyl-xylobiose.

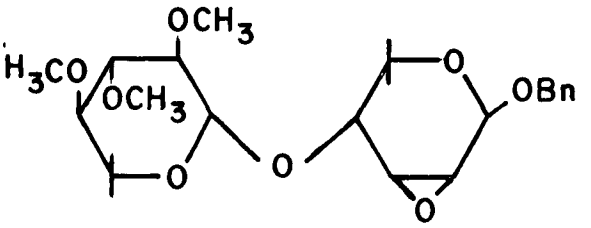
Based on the product analyses, the presence of the methoxyl substituents in 2',3',4'-tri-O-methyl-xylobiose increases the amount of fragmentation products from the reducing end-unit relative to xylobiose. In addition, for both disaccharides

low alkalinity increases the amount of fragmentation products, whereas, increased ionic strength decreases the amount of fragmentation products relative to D-xyloisosaccharinic acid.

Based on the results of the disaccharide syntheses, silver oxide is a better promoter for the Koenigs-Knorr condensation of XXXVI and XXIII to form benzyl 2,3-anhydro-4-O-(β-D-xylopyranosyl)-β-D-ribopyranoside (XXV) than silver trifluoromethanesulfonate. In addition, hydroxide ion is not stereospecific in opening the epoxide of XXV as earlier work indicated (46); disaccharide products with xylo- and arabino- configuration in the reducing end-group were formed in a ratio of nine to one, respectively. In contrast, benzyl oxide ion is highly stereoselective for opening the epoxide of benzyl 2,3-anhydro-4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-ribopyranoside (XXVII) yielding primarily (96%) the disaccharide product with the xylo- configuration in the reducing end-group; hydroxide ion produced approximately equal amounts of the xylo- and arabino-configuration.



XXV



XXVII

EXPERIMENTAL

GENERAL

Melting points were determined on a Thomas Hoover capillary apparatus which had been calibrated against known compounds.

Elemental analyses were performed by Chemalytics, Inc., 2330 S. Industrial Park Dr., Tempe, Arizona.

Optical rotations were measured with a Perkin-Elmer 141 MC polarimeter.

Thin-layer chromatography (TLC) was performed on microscope slides coated with silica gel G (Brinkman Instruments, Inc.). The compounds were detected by spraying the developed chromatogram with methanolic sulfuric acid (20%, w/w) with subsequent charring for visualization. Developing solvents were as follows: Solvent 1 - ethyl acetate; Solvent 2 - chloroform:ethyl acetate, 4:1, v/v; Solvent 3 - chloroform:methanol, 3:1, v/v; Solvent 4 - chloroform:methanol, 2.5:1, v/v; Solvent 5 - chloroform:methanol, 2:1, v/v; Solvent 6 - chloroform: methanol, 15:1, v/v.

Gas-liquid chromatographic (GLC) analyses were conducted on a Varian Aerograph 2700 gas chromatograph equipped with a hydrogen flame-ionization detector and a Varian A-25 recorder with a Disc integrator. All columns were rigged for on-columm injection, and made of 0.125 inch O.D. stainless steel unless otherwise noted. The conditions were as follows:

Condition A: 3% SP-2340 on 100-120 mesh Supelcoport (10 ft); nitrogen, 30 mL/ min; injector, 260°; oven, 205°; detector, 260°.

Condition B: 3% SE-30 on 100-120 mesh Gas Chrom Q (5 ft); nitrogen, 30 mL/min; injector, 255°; oven, 150→194° at 2° min^{-1}, 194→230° at 6° min^{-1}, and hold; detector, 270°.

Condition C: 3% SE-30 on 100-120 mesh Chromsorb W-HP (5 ft); nitrogen, 30 mL/min; injector, 250°; oven, 145→230° at 4°min^{-1}; detector, 250°.

Condition D: 3% SP-2250 on 100-120 mesh Supelcoport (glass, 14 ft x 0.125 inch i.d.); oven, 60→200° at 2°min^{-1}; detector, 230°; injector, 230°.

Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were determined using a Joel FX-100 spectrometer at normal probe temperature. Tetramethylsilane was used as the internal standard in organic solvents. When deuterium oxide was used as the solvent p-dioxane, set relative to TMS at 67.4 PPM, was used as the internal standard.

Mass spectra were determined on a DuPont Instruments 21-491 mass spectrometer interfaced via a jet separator with a Varian Aerograph 1400 gas chromatograph equipped with a hydrogen flame ionization detector. The chromatographic response was recorded on a Hewlet-Packard 7128A recorder; mass spectra were recorded on a Century GPO 460 oscillographic recorder.

Chromatographic separations were performed on a 3% OV-17 column (80-100 mesh Supelcoport, 15 ft x 0.125 inch, stainless steel). The following conditions were employed: helium (UHP, 99.999%, Matheson Gas Products), 30 mL/min; oven, 45→250° at 2°min^{-1}; injector, 260°; detector, 260°.

Control settings for the mass spectrometer were; oven, 100°; source, 180°; sensitivity, 4-10; ionizing voltage, 70 eV; scan, 40 sec/decade; chart speed, 4 inch/sec; interface block, 300°; connecting tube, 280°; jet separator, 250°.

SOLVENTS, SOLUTIONS, AND CATALYSTS

BENZENE

Benzene (1L) was refluxed over phosphorus pentoxide (20 g) for 1 hour and fractionally distilled (40 cm Vigreux column) with the exclusion of moisture. The fraction boiling between 78-79°C was retained and stored in sealed bottles.

PALLADIUM ON CHARCOAL CATALYST (10%)

The catalyst was prepared as described by Mozingo (60) and stored in a desiccator over sodium hydroxide.

SILVER OXIDE

The catalyst was prepared as described by Millard (61).

SODIUM HYDROXIDE STOCK SOLUTION

Triply distilled water was boiled for 1 hour, then cooled to room temperature. Reagent grade sodium hydroxide (450 g) pellets were added immediately to the cooled water (450 g), the solution cooled, filtered, and placed in a stoppered, paraffin-lined glass bottle.

PREPARATION OF COMPOUNDS AND PROOF OF STRUCTURE

BENZYL β-D-ARABINOPYRANOSIDE (XXXII)

The glycoside (XXXII) was prepared using a slight modification of the procedure outlined by Ballou (62). D-Arabinose (100 g) and benzyl alcohol (500 mL) were cooled in an ice-salt bath for 30 minutes. Acetyl chloride (50 mL) was added dropwise over 1 hour while maintaining vigorous stirring. The solution

was stirred for an additional 30 min., removed from the ice-salt bath, and stirred until TLC (Solvent 1) indicated the reaction had reached completion (ca. 12 hr). The gelatinous product mixture was diluted with ethyl ether (1 L) and cooled in an ice-salt bath to promote crystallization. The crystals were filtered, washed with absolute ethanol (200 mL), and dried at room temperature in a vacuum oven. The crude product was recrystallized from water to yield pure glycoside (XXXII)(135 g, 84%), mp 169.5-170.5°, $[\alpha]_D^{18}$ -212° (c 0.379, water). Literature (63): mp 169-171°, $[\alpha]_D^{25}$ -212° (c 0.5, water).

Compound XXXII did not reduce Fehlings solution, which is indicative of a glycosidic linkage. The ^{13}C-NMR spectrum of XXXII in DMSO-d_6 (Fig. 20, Appendix I) confirms the presence of the benzyl group. Resonances at 138 and 127-128 ppm confirm the presence of aromatic carbons associated with the benzyl group (64).

BENZYL 3,4-O-ISOPROPYLIDENE-β-D-ARABINOPYRANOSIDE (XXXIII)

This derivative (XXXIII) was prepared by the procedure described by Buchanan, Clode, and Vethaviyasar (65) for the L-anomer. A mixture of XXXII (113 g), 2,2-dimethoxypropane (400 mL), dry acetone (400 mL), and p-toluenesulfonic acid (4 g) was heated on a steam bath until all the solids were dissolved. TLC (Solvent 2) indicated the reaction was complete after 1 hour of heating. The solution was cooled to room temperature, neutralized with potassium carbonate, filtered and evaporated in vacuo to a pale yellow sirup. The sirup was dissolved in ethyl ether (300 mL), and unreacted XXXII was removed by filtration. The filtrate was concentrated in vacuo to a sirup which crystallized on standing. Recrystallization from petroleum ether-ethyl ether (15:1, v/v) afforded pure XXXIII (126 g, 96%), mp 56.5-58°, $[\alpha]_D^{20}$ -206.9° (c 1.07, ethanol). Literature (62): mp 55-58°•$[\alpha]_D$ -209°(c 2.0, ethanol).

The ^{13}C-NMR spectrum of XXXIII in chloroform-d (Fig. 21, Appendix I) confirmed the presence of the isopropylidene group. A resonance at 109 ppm was indicative of the acetal carbon while two resonances at 26 and 28 ppm were indicative of the two methyl groups (66).

BENZYL 3,4-O-ISOPROPYLIDENE-2-O-p-TOLUENESULFONYL-β-D-ARABINOPYRANOSIDE (XXXIV)

Compound XXXIV was prepared using the method outlined by Buchanan, et al. (65) for the L-derivative. The isopropylidene derivative (XXXIII) was heated at 50°C with pyridine (400 mL) containing p-toluenesulfonyl chloride (115 g) until TLC (Solvent 2) indicated the reaction was complete (ca. 5 hr). The solution was allowed to cool and the crystalline material was removed by filtration. Water (10 mL) was slowly added to the filtrate to decompose the excess p-toluenesulfonyl chloride and the filtrate was extracted with chloroform. The chloroform solution was washed with 2M sulfuric acid (2 x 500 mL) and water (2 x 500 mL), dried over calcium sulfate, and evaporated to a thick brown sirup. Crystallization of the sirup from ethanol:water (9:1, v/v) gave pure XXXIV (130 g, 88%), mp 92-94°, $[\alpha]_D^{22}$ -184.1° (c 0.95, chloroform). Literature (67): mp 96°, $[\alpha]_D^{25}$ -187° (c 0.5, chloroform).

The ^{13}C-NMR spectrum of XXXIV in acetone-d_6 (Fig. 22, Appendix I) confirmed the presence of the p-toluenesulfonyl group. A single resonance at 21.4 ppm was indicative of the methyl carbon while resonances at 145.5 and 134.1 ppm were characteristic of aromatic carbons (64).

The crystalline material that was initially filtered from the pyridine solution was recrystallized from isopropyl ether to yield white crystals (10 g), mp 123-123.5°, $[\alpha]_D^{22}$ -158.8° (c 0.79, chloroform). The physical constants as well as TLC (Solvent 2) and NMR analyses were identical with

those of XXXV. The mixed melting point of these crystals and pure XXXV was undepressed. This information indicates that some benzyl β-D-arabinopyranoside (XXXII) was present in the reaction and that it was selectively esterfied at the C-2 position. However, subsequent attempts to selectively esterify XXXII produced a series of partially esterified derivatives in almost equal yield.

BENZYL 2-O-p-TOLUENESULFONYL-β-D-ARABINOPYRANOSIDE (XXXV)

Procedures outlined by Buchanan, et al. (65) were used to prepare XXXV. A mixture of XXXIV (130 g), aqueous ethanol (80%, v/v,1 L), and IR-120 ion exchange resin (100 mL) was refluxed until TLC (Solvent 2) indicated the reaction was complete (ca. 2 hr). The solution was cooled, filtered, and the filtrate was concentrated in vacuo to yield crystals. The product was washed with water and dried in a vacuum oven. Recrystallization from isopropyl ether: ethanol (30:1, v/v) gave pure XXXV(89 g, 76%), mp 123-123.5°, $[\alpha]_D^{20}$ -156.8° (c 0.782, chloroform). Literature (68): mp 123.5-124.5°, $[\alpha]_D^{20}$ -156° (c 0.8, chloroform).

The ^{13}C-NMR spectrum of XXXV in chloroform-d (Fig. 23, Appendix I) confirmed the removal of the isopropylidene group. In addition to the lack of characteristic isopropylidene carbon resonances at 109, 28, and 26 PPM, the C-3 and C-4 resonances moved down-field to 67.2 and 69.6 PPM which is in the region of hydroxyl bearing carbons.

BENZYL 2,3-ANHYDRO-β-D-RIBOPYRANOSIDE (XXIII)

This derivative (XXIII) was prepared using a modification of the procedure described by Buchanan, et al. (65). A solution of XXXV (190 g) in methanolic sodium methoxide (0.5N, 1.5L) was heated at 50°C until TLC (Solvent 2) indicated the reaction was complete. The solution was evaporated in vacuo to 500 mL and

neutralized with sulfuric acid. Care was taken at this point to ensure the solution did not become acidic because of the acid sensitivity of the epoxide bond. The epoxide XXIII crystallized from the solution with cooling, and was filtered, washed with water, and dried in vacuo. Recrystallization from isopropyl ether yielded pure XXIII (71 g, 75%), mp 76–77.5°, $[\alpha]_D^{22}$ -65.1° (c 0.78, chloroform). Literature (68): mp 76–77°, $[\alpha]_D^{20}$ -67° (c 0.8, chloroform).

The ^{13}C-NMR spectrum of XXIII in chloroform-d (Fig. 24, Appendix I) confirmed the presence of an epoxide bond and the removal of the p-toluenesulfonyl group. The presence of the epoxide bond attached at the C-2 and C-3 carbons shifted the resonances of these carbons upfield to form a single peak at 51.9 PPM. Removal of the p-toluenesulfonyl group was indicated by the lack of aromatic resonances at 145.5 and 134.1 PPM, and the methyl resonance at 21.4 PPM which are characteristic of this functional group.

2,3,4-TRI-O-ACETYL-α-D-XYLOPYRANOSYL BROMIDE (XXXVI)

Compound XXXVI was prepared as described by Schroeder, et al. (69). A solution of 1,2,3,4-tetra-O-acetyl-β-D-xylopyranose (20 g) in 1,2-dichloroethane (40 mL) was reacted with hydrogen bromide in acetic acid (50 mL, 41%, v/v) for 4 hrs. The solution was poured into water (1 L), and extracted with chloroform (150 mL). The chloroform layer was dried over calcium chloride and evaporated in vacuo until the first crystals appeared. Two recrystallizations from isopropyl ether gave pure XXXVI (15 g, 55%), mp 99–101°. Literature (69): mp 101–102°. This compound is very sensitive to moisture and heat.

BENZYL 2,3-ANHYDRO-4-O-(β-D-XYLOPYRANOSYL)-β-D-RIBOPYRANOSIDE (XXV)

A mixture of XXIII (4 g), silver oxide (13.5 g), 10-20 mesh Drierite (25 g), and dry benzene (65 mL) was stirred in the dark for 16 hours. Addition of iodine (1.5 g) was followed by addition (1 mL min^{-1}) of XXXVI (7 g) in dry benzene. The stirring was continued until TLC (Solvent 2) indicated the reaction was complete (ca. 6 days). The solution was filtered through celite, and the residue was washed with benzene. The filtrate was concentrated in vacuo and the sirup was deacetylated in dry methanol (100 mL) containing sodium methoxide (0.5N, 5 mL) with heating. Cooling the mother liquor promoted crystallization of TLC (Solvent 3) pure XXV (3.33 g, 52.7%). Recrystallization from methanol provided pure XXV; mp 202.5-203°, $[\alpha]_D$ -38.6° (c 0.994, dimethyl sulfoxide). $C_{17}H_{22}O_8$ requires C, 57.6; H, 6.2. Found C, 57.3; H, 5.93%.

The ^{13}C-NMR spectrum of XXV in DMSO-d_6 (Fig. 26, Appendix I) confirmed the presence of the xylose moiety. A new resonance at 103.5 PPM is indicative of C-1' carbons attached via a glycosidic linkage to another sugar substituent (66). The additional resonances at 76.5-65.6 PPM are all typical of resonances associated with a xylose moiety (66).

Compound XXV was also prepared using the method described by Hanessian, et al. (70) for other compounds. Tri-O-acetyl-α-D-xylopyranosyl bromide (2.6 g) in dry dichloromethane (45 mL) was treated successively with 1,1,3,3-tetramethyl urea (1.5 mL), silver trifluoromethanesulfonate (2.5 g), and XXIII (1 g). The mixture was allowed to react in the dark for 3 days at 0°C. An additional quantity of XXXVI (1 g) was added on the second day. The solution was filtered through celite, the celite washed with dry dichloromethane and the combined extracts washed with aqueous sodium hydrogen carbonate. The dichloromethane solution was dried over drierite and the solvent removed by evaporation

in vacuo leaving a sirup. The sirup was purified by using a dry pack silica gel (130 g, 60-200 mesh) column (1 m x 2.5 cm) with chloroform as the eluant. The pure fractions were collected, the solvent removed by evaporation *in vacuo* and the resulting sirup dissolved in methanol (40 mL). XXIV was deacetylated by adding sodium methoxide (0.5N, 0.5 mL) to the methanol solution and warming on a steam bath (*ca.* 5 min). The resulting crystals were filtered, washed with cold methanol (50 mL), and dried to produce pure XXV (0.59 g, 37.2%).

BENZYL β-XYLOBIOSIDE (XXVI)

Disaccharide (XXVI) was obtained by reacting XXV (1 g) in aqueous sodium hydroxide (2N, 100 mL) at a temperature just below reflux under a nitrogen atmosphere. TLC (Solvent 4) indicated the reaction was complete after 7 hrs. The solution was cooled, deionized with MB-3 resin, the resin washed with hot water, and the sample dried to a hard sirup (1 g, 95%) which failed to crystallize. Further drying yielded a hygroscopic powder, $[\alpha]_D^{23}$ -77.3° (c 0.225, water). $C_{17}H_{24}O_9$ requires C, 54.8; H, 6.45. Found C, 54.6; H, 6.37%.

The ^{13}C-NMR spectrum of XXVI in DMSO-d_6 (Fig. 28, Appendix I) confirmed that the epoxide bond of XXV was opened. The characteristic carbon resonances associated with the epoxide bond at 53.4 and 51.9 PPM were absent, while two resonances appeared at 76.1-69.4 PPM which are in the region where hydroxyl bearing carbons appear.

BENZYL β-XYLOBIOSIDE PENTAACETATE (XXXVII)

Crude XXVI (1.1 g) was reacted with acetic anhydride:pyridine (35 mL, 3:4, v/v) until TLC (Solvent 2) indicated the reaction was complete (*ca.* 16 hrs). The solution was stirred with ice (100 mL) for 0.5 hr, and extracted with chloroform (100 mL). The chloroform extract was washed with 1N hydrochloric

acid (100 mL), dried ($CaSO_4$), and concentrated *in vacuo* to a sirup. Crystallization from isopropyl ether yielded XXXVII (1.4 g, 75%); mp 126.3-127°, $[\alpha]_D$ -102.9° (c 0.212, chloroform). $C_{27}H_{34}O_{14}$ requires C, 55.7; H, 5.9. Found C, 55.6; H. 5.9%.

XYLOBIOSE (XXI)

Compound XXI was prepared by the method as described by Aspinall and Ross (46) for a partially methylated analog. A solution of XXVI (4 g) in aqueous ethanol (85%, v/v, 100 mL) was stirred with 10% palladium-on-carbon catalyst (1 g) while maintaining a hydrogen atmosphere (slight positive pressure). TLC (Solvent 5) indicated the reaction was complete after 2 days. The solution was filtered through celite, and the celite was washed successively with hot water (50 mL) and hot ethanol (50 mL). The filtrate was concentrated *in vacuo* to a sirup (2 g, 66%). Acid hydrolysis, sodium borohydride reduction, acetylation, and GLC analysis (Condition A) of a portion of the crude product indicated the reducing end contained 10% arabino- and 90% xylo- configuration.

Pure XXI (0.7 g, 84%) was obtained by deacetylating purified β-xylobiose hexaacetate (XXXVIII) (1.6 g) in acetone-dry methanol (4:1, v/v, 60 mL) with sodium methoxide (0.5N, 0.5 mL) at 0°C. The deacetylation process was complete after 4 weeks providing pure XXI as a white powder. Pure XXI was filtered, the powder washed with acetone, dried, and placed in a desiccator over phosphorous pentoxide. $[\alpha]_D^{31}$ -28.1 → 25.3° (c 1.0, H_2O). Literature (79): $[\alpha]_D^{25}$ -32.0 → 25.5° (c 2-3.0, H_2O).

The ^{13}C-NMR spectrum of XXI in D_2O (Fig. 7, Appendix I) confirmed the removal of the benzyl substituent. The characteristic aromatic resonances at 137.8, 128.0, and 127.4 PPM were absent while two new resonances appeared at 97.3 and

92.8 PPM. These resonances are in the region where α and β anomeric carbons are found (66).

β-XYLOBIOSE HEXAACETATE (XXXVIII)

The acetate derivative (XXXVIII) was prepared as described by Whistler, et al. (71). Impure XXI (0.6 g) was mixed with acetic anhydride (9 mL), acetic acid (1 mL), and sodium acetate (1 g), and heated on a steam bath until TLC (Solvent 2) indicated the reaction was complete (ca. 1.5 hr). The solution was cooled, stirred (ca. 15 min) with ice-water (100 mL), and extracted with chloroform (2 x 100 mL). The chloroform extracts were dried ($CaSO_4$) and concentrated in vacuo to a sirup. The sirup was crystallized from ethyl acetate by addition of low boiling petroleum ether and seeding. Three recrystallizations afforded TLC (Solvent 2) pure XXXVIII (0.8 g, 69.6%); mp 154-155°, $[\alpha]_D$ -75.2° (c 1.5, chloroform). Literature (71): mp 155-156°, $[\alpha]_D$ -75.1° (c 10.0, chloroform).

The ^{13}C-NMR spectrum of XXXVIII in acetone-d_6 (Fig. 6, Appendix I) confirmed that XXI was acetylated. The characteristic resonances associated with the α and β C-1 carbons at 97.3 and 92.8 PPM were absent while a new resonance appeared at 92.7 PPM. This peak is in the region where the β-acetate anomer resonance is expected (66).

BENZYL 2,3-ANHYDRO-4-O-(2,3,4-TRI-O-METHYL-β-D-XYLOPYRANOSYL)-β-D-RIBOPYRANOSIDE (XXVII)

A modification of a procedure described by Schroeder, et al. (72) for the methylation of 3,4,6-tri-O-acetyl-1,2-O-(exo-ethoxy)ethylidene-α-D-glucopyranose was used in the synthesis of XXVII. Dimethyl sulfate (10 mL) was added dropwise over a 2 hour period with continuous stirring to a solution of XXV (1 g) in tetrahydrofuran (50 mL) over powdered sodium hydroxide (3 g). After

TLC (Solvent 2) indicated the reaction was completed (ca. 12 hrs) the excess dimethyl sulfate was destroyed by dropwise addition of water (20 mL) with thorough agitation for 1 hr. The tetrahydrofuran was removed in vacuo leaving the water layer and a sirupy residue. The aqueous mixture was extracted with cloroform (2 x 50 mL), and the chloroform extracts were dried ($CaSO_4$), and concentrated in vacuo to a crystalline mass. Recrystallization from low boiling petroleum ether-ethyl acetate (5:1, v/v) provided pure XXVII (0.7 g, 67%); mp 103-104°, $[\alpha]_D$ -44.9° (c 0.932, chloroform). $C_{20}H_{28}O_8$ requires C, 60.6; H, 7.0. Found C, 60.6; H, 7.2%. The mother liquor containing partially methylated components was concentrated in vacuo to a sirup then remethylated.

The ^{13}C-NMR spectrum of XXVII in acetone-d_6 (Fig. 30, Appendix I) confirmed that the free hydroxyl groups were fully methylated. Three new resonances appeared at 86.1, 84.2, and 80.3 PPM which are typical of methoxyl bearing carbons. In addition, three new resonances appeared at 60.2, 60.1, and 58.5 PPM which are in the region of methoxyl carbon resonances. The off resonance spectrum of this region indicates that the methoxyl carbons form quartets.

BENZYL 3-O-BENZYL-2',3',4'-TRI-O-METHYL-β-XYLOBIOSIDE (XXVIII)

Compound XXVIII was prepared as described by Doerr, et al. (47) for the synthesis of a nucleoside. Compound XXVII (0.3 g) was dissolved in benzyl alcohol (10 mL) containing sodium metal (0.1 g) and heated at 60° C (ca. 3 hrs). The cooled solution was diluted with methanol (100 mL) and treated with IR-120 ion exchange resin (50 mL). The solution was filtered, and the resin was washed with hot methanol (10 x 25 mL). The filtrate was concentrated in vacuo with the benzyl alcohol being removed as its aqueous azeotrope. The product was a yellow sirup (0.367 g, 97%); $[\alpha]_D$ -77.3° (c 1.7, chloroform).

The ^{13}C-NMR spectrum of XXVIII in acetone-d_6 (Fig. 31, Appendix I) indicated that the epoxide of XXVII was opened. In addition, new resonances at 140.0, 127.8, and 127.4 PPM confirm the presence of aromatic carbons associated with a second benzyl group. The characteristic resonances associated with the epoxide of XXVII at 52.3 and 53.5 PPM were absent, while two new resonances appeared at 82.4 and 73.6 PPM. The resonance at 73.6 PPM is in the region of hydroxyl bearing carbons, while the resonance at 82.4 PPM is in the region where etherified carbons appear (66).

2',3',4'-TRI-O-METHYL-β-XYLOBIOSE (XXII)

Partially methylated xylobiose was prepared from XXVIII using the procedure described earlier for preparation of xylobiose from XXVI. Compound XXVIII (1.3 g) in aqueous ethanol (85%, v/v, 80 mL) was stirred for 3.5 days with 10% palladium-on-carbon catalyst (1 g) while maintaining a hydrogen atmosphere (slightly positive pressure). After TLC (Solvent 6) indicated the reaction was complete the mixture was filtered through celite, and the celite was washed successively with hot methanol (100 mL) and hot chloroform (100 mL). The filtrate was concentrated in vacuo to a sirup (0.76 g, 88%); $[\alpha]_D^{31}$ -25.4 → -33.8° (c 0.8, H_2O)• $C_{20}H_{30}O_9$ requires C, 48.1; H, 7.4. Found C, 47.9; H, 7.4%.

Hydrolysis, reduction, acetylation, and GLC analysis (Condition A) of a portion of the product showed that the reducing end contained 3-4% arabino- and 96-97% xylo- configuration.

The ^{13}C-NMR spectrum of XXII in DMSO-d_6 (Fig. 8, Appendix I) confirmed the removal of the two benzyl substituents. The characteristic aromatic resonances at 140-127 PPM were absent while two new resonances appeared at 97.5 and 92.2 PPM. These resonances are in the region where α and β anomeric carbons are found (66).

2,3,4-TRI-O-METHYL-D-XYLOSE (XXXIX)

The procedure described by Schroeder, et al. (72) for the methylation of 3,4,6-tri-O-acetyl-1,2-O-(exo-ethoxy)ethylidene-α-D-glucopyranose was used in the preparation of XXXIX. Dimethyl sulfate (50 mL) was added dropwise over 3 hr with continuous stirring to a mixture of powdered sodium hydroxide (60 g) and methyl α-D-xyloside (10 g) in tetrahydrofuran (350 mL). TLC (Solvent 2) indicated the reaction was complete after 19 hrs. The excess methylating reagent was destroyed by the dropwise addition of water (100 mL) with vigorous agitation (ca. 1 hr) and subsequent heating (65° for 1 hr). The solvent was removed in vacuo and the residue was extracted with chloroform (150 mL). The chloroform extract was dried ($CaSO_4$) and concentrated in vacuo to a sirup (11 g). The sirup was refluxed for 11 hrs in 1N HCl (100 mL). The solution was cooled, neutralized with barium carbonate, and filtered. The filtrate was extracted with chloroform, and the extract was dried ($CaSO_4$) and concentrated in vacuo to a sirup. Crystallization of the sirup from isopropyl ether afforded TLC (Solvent 2) pure XXXIX (6 g, 50.7%); $[\alpha]_D$ + 14° → + 19.9° at equilibrium (c 2.0, water); Literature (73): $[\alpha]_D$ + 17 at equilibrium (c 2.0, water).

The low yield of XXXIX was due to the volatility of the intermediate and end products.

KINETIC STUDIES

PREPARATION OF OXYGEN FREE SODIUM HYDROXIDE

Triply-distilled water was boiled for 1 hr and allowed to cool to room temperature while being purged with nitrogen. The sodium hydroxide reaction solution was prepared in a glove bag that had been evacuated and filled with

nitrogen (cycle repeated 3 times). A sodium hydroxide solution of slightly greater than the desired normality was prepared using the stock sodium hydroxide solution and triply-distilled water. The solution was titrated against potassium acid phthalate. An appropriate quantity of the sodium hydroxide solution was weighed into a volumetric flask and diluted with oxygen-free triply-distilled water. The final solution was titrated against potassium acid phthalate.

When constant ionic strength studies were conducted an appropriate quantity of sodium acetate was weighed into the volumetric flask of the final solution. The other manipulations were the same as described earlier.

LOADING THE REACTION TUBES

A volumetric flask containing the disaccharide (0.0012M or 0.0015M), internal standard, and triply-distilled water was placed in the glove bag along with the test tube rack, reaction vessels, and the appropriate solutions. The glove bag was closed, and evacuated and filled with prepurified nitrogen (cycle repeated 3 times). The sugar solution (1 mL) was pipetted into the sidearm of the reaction vessel (See Fig. 19) and the sodium hydroxide solution (25 mL) was pipetted into the large portion of the reactor. The tubes were sealed with teflon lined screw caps. The rack was removed from the glove bag and placed in a water bath at 30°C ± 0.1° to equilibrate for 1 hr.

INITIATION OF THE REACTION AND SAMPLING

The rack was removed from the water bath, inverted and manipulated in a manner to thoroughly mix the contents of the two compartments of the tubes. Time zero was taken to be the midpoint of this process. The rack was then placed back into the water bath. Samples were removed from the water bath at appropriate intervals

and quenched with 0.5M boric acid (180 mL) and reduced with sodium borohydride (0.5 g) for a 12 hr period.

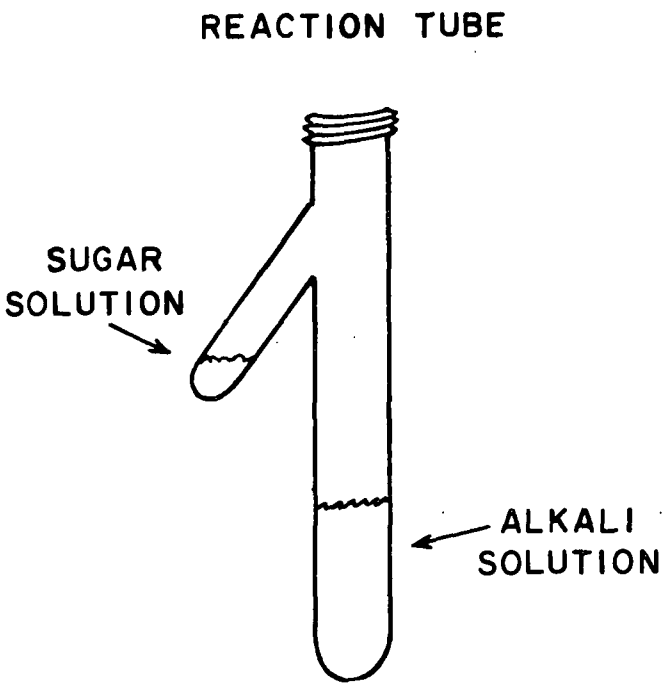


Figure 19. Reaction Vessel

SAMPLE WORKUP PROCEDURE

Each sample was stirred for 30 min with prewashed IR-120 resin (70 mL). The resin was removed by filtration, washed with distilled water (100 mL) and the combined filtrate was evaporated in vacuo to a white powder. The sodium borate was removed as volatile methyl borate by three additions of methanol (80 mL) and subsequent evaporation in vacuo.

The residue was dissolved in water (100 mL) and passed through a bed of MB-3 resin (30 mL). The resin was washed with water (3 x 33 mL), and the combined filtrate was concentrated in vacuo to a small volume. The solution was transferred to a 10 mL Erlenmeyer flask and concentrated in vacuo to dryness.

The dried sample was acetylated by shaking the sample with an acetic anhydride-pyridine solution (0.5 mL, 1:1, v/v) for 12 hrs. The acetylation solution was used directly in the gas chromatographic analysis. Condition B was used for xylobiose and Condition C for 2',3',4'-tri-O-methyl-xylobiose.

PRODUCT ANALYSIS

The neutral and acidic products of the degradation reactions were identified by GLC-MS and measured semiquantitatively by GLC employing the trimethylsilyl (TMS) derivatives. The TMS derivatives were prepared using a two phase system employing dimethylsulfoxide and Tri-Sil Concentrate (Pierce Chemical Co.).

An aliquot of the reaction solution (5 mL) was stirred with IR-120 resin (20 mL). The resin was filtered and washed with water (50 mL). The filtrate was stirred with 0.5M ammonium hydroxide (20 mL) for 30 min and then concentrated to dryness in 4 mL vials. The last traces of water were removed as an azeotrope of 1,2-dichloroethane. The dried sample was dissolved in dimethylsulfoxide (0.2 mL) by heating at 60°C for 30 min and then treated with Tri-Sil Concentrate (0.2 mL). The two layer system was heated at 60°C for 1 hr and shaken mechanically for 12 hrs. Samples of the top layer were injected directly in the gas chromatograph (Condition D).

NOMENCLATURE

GLC	gas-liquid chromatography
TLC	thin-layer chromatography
MS	mass spectrometry
GLC-MS	gas-liquid chromatography-mass spectrometry
M	molar, moles/liter
N	normality, equivalents/liter
TMS	trimethylsilyl derivative
mp	melting point
w/w	weight to weight ratio
v/v	volume to volume ratio
^{13}C-NMR	^{13}carbon nuclear magnetic resonance spectroscopy
m/e	mass to charge ratio (mass spectroscopy)
$[\alpha]_D$	specific optical rotation at 589 nm
TMSiOH	trimethylsilanol

ACKNOWLEDGMENTS

The author thankfully acknowledges the help and encouragement of his Thesis Advisory Committee consisting of Drs. L. R. Schroeder, N. S. Thompson, D. R. Dimmel, and R. D. McKelvey. In particular, the author thanks Dr. Schroeder for his many ideas, constructive criticism, and his friendship. The help and suggestions of Messrs. M. C. Filz and P. E. Van Rossum in development of the reactor assembly is appreciated. The author wishes to express his thanks to other staff and faculty, too numerous to mention, who have helped during the course of his studies.

The author thanks John Gast for the ^{13}C-NMR analyses and his help in the interpretation of the ^{13}C-NMR spectra.

The financial support of The Institute of Paper Chemistry is deeply appreciated.

The author also wishes to thank his fellow students and their wives for making our stay at the Institute very memorable.

I want to thank our families for their encouragement and prayers during the course of the thesis work. Finally, I want to thank my wife, Barbara, for her patience, prayers, personal sacrifice, and most of all her love.

LITERATURE CITED

1. Wollwage, J. C., Tappi 59(4):54(1976).
2. Kleppe, P. J., Tappi 53(1):35(1970).
3. Proctor, A. R. and Wiedenkamp, R. H., J. Polymer Sci. Part C 28:1 (1969).
4. Ahlgren, P., Ishizu, A., Szabo, I., and Theander, O., Svensk Papperstid. 71:355(1968).
5. Landucci, L. L. and Sanyer, N., Tappi 57(10):97(1974).
6. Brunn, H., Gadda, L., and Storsjo, M., Tappi 62(4):65(1979).
7. Meller, A., Tappi 48:231(1965).
8. Whistler, R. L. and BeMiller, J. N., Adv. Carbohyd. Chem. 13:289(1958).
9. Isbell, H. S., J. Res. Natl. Bur. Std. 29:227(1942)
10. Samuelson, O. and Franzon, D., Svensk Papperstid. 60:872(1957).
11. Alfredsson, B., Gedda, L., and Samuelson, O., Svensk Papperstid. 64:694(1961).
12. Lai, Y. Z. and Sarkanen, K. V., J. Polymer Science, Part C 28:15(1969).
13. Young, R. A., Sarkanen, K. V., Johnson, P. G., and Allen, G. G., Carbohyd. Res. 21:111(1972).
14. Johansson, M. H. and Samuelson, O., Carbohyd. Res. 34:33(1974).
15. Kenner, J. and Richards, G. N., J. Chem. Soc. 1953:2240.
16. Kenner, J. and Corbett, W. M., J. Chem. Soc. 1953:2245.
17. Kenner, J. and Richards, G. N., J. Chem. Soc. 1954:278.
18. Kenner, J. and Richards, G. N., J. Chem. Soc. 1954:1784.
19. Kenner, J. and Corbett, W. M., J. Chem. Soc. 1954:1789.
20. Kenner, J. and Corbett, W. M., J. Chem. Soc. 1954:3274.
21. Kenner, J. and Corbett, W. M., J. Chem. Soc. 1954:3277.
22. Kenner, J. and Richards, G. N., J. Chem. Soc. 1954:5366.
23. Kenner, J. and Corbett, W. M., J. Chem. Soc. 1954:3281.
24. Kenner, J. and Corbett, W. M., J. Chem. Soc. 1955:1431.
25. Kenner, J., Corbett, W. M., and Richards, G. N., J. Chem. Soc. 1955:1709.

26. Kenner, J. and Richards, G. N., J. Chem. Soc. 1955:1810.

27. Kenner, J. and Richards, G. N., J. Chem. Soc. 1956:2916.

28. Kenner, J. and Richards, G. N., J. Chem. Soc. 1956:2921.

29. Kenner, J. and Corbett, W. M., J. Chem. Soc. 1957:927.

30. Kenner, J. and Richards, G. N., J. Chem. Soc. 1957:3019.

31. Blears, M. J., Machell, G., and Richards, G. N., Chem. and Ind. 1957:1150.

32. Aspinall, G. O., Carter, M. E., and Los, N., J. Chem. Soc. 1956:4807.

33. MacLaurin, D. J. and Green, J. W., Can. J. Chem. 47:3957 (1969).

34. Aspinall, G. O., Greenwood, C. T., and Sturgeon, R. J., J. Chem. Soc. 1961:3667.

35. MacLeod, J. M., Anaerobic alkaline degradation of D-glucose, cellobiose, and derivatives. Doctoral Dissertation. Appleton, Wisconsin, The Institute of Paper Chemistry, 1975.

36. Sten, M. and Mustola, T., Cellulose Chem. Technology 7:359(1973).

37. Machell, G. and Richards, G. N., J. Chem. Soc. 1960:1932.

38. Machell, G. and Richards, G. N., J. Chem. Soc. 1960:1938.

39. Malinen, R. and Sjostrom, E., Cellulose Chem. and Technol. 9:651(1975).

40. Lowendahl, L. and Samuelson, O., Acta Chem. Scand. B 30:691(1976).

41. Johansson, M. H. and Samuelson, O., Chemica Scripta. 9:151(1976).

42. Rao, V. S. R. and Foster, J. F., Biopolymers 1:527(1963).

43. Schroeder, L. R., personal communication.

44. Young, R. A. and Liss, L., Cellulose Chem. Technol. 12:399(1978).

45. Williams, N. R., Advances in Carbohydrate Chemistry and Biochemistry 25:109-179(1970).

46. Aspinall, G. O. and Ross, K. M., J. Chem. Soc., Sept. 1961:3674.

47. Doerr, I. L., Cadington, J. F., and Fox, J. J., J. Org. Chem. 30:467(1965).

48. Green, J. W., Pearl, I. A., Hardacker, K. W., Andrews, B. D., and Haigh, F. C., Tappi 60(10):120(1977).

49. Lindstrom, L. A. and Samuelson, O., Acta Chem. Scand. B 31:479(1977).

50. Ziderman, I. and Bel-Ayche, J., J. of Applied Polymer Sci. 22:1151(1978).

51. Brandon, R. E., Alkaline degradation of 1,5-anhydrocellobiitol. Doctor's Dissertation, Appleton, Wis., The Institute of Paper Chemistry, 1971.

52. Wylie, T. R. The alkaline degradation of 1,5-anhydro-2,3,6-tri-O-methyl-cellobiitol. Doctor's Dissertation, Appleton, Wis., The Institute of Paper Chemistry, 1979.

53. Kiss, J., Adv. Carbohyd. Chem. 29:229(1974).

54. Harned, H. S. and Owen, B. B., Physical Chemistry of Electrolytic Solutions. Reinhold Publishing Company, 1958, New York, 615 p.

55. Gould, E. S., Mechanism and Structure in Organic Chemistry. Holt, Rinehart and Winston, 1959, New York, 187 p.

56. Laurent, T. C., J. Am. Chem. Soc. 78:1875(1956).

57. Laurent, T. C. and Wertheim, E. M., Acta Chem. Scand. 6:678(1952).

58. Pigman, W. and Isbell, H. S., Adv. Carbohyd. Chem. 23:38(1968).

59. Eastham, A. M., Blackall, E. L. and Latremouille, G. A., J. Am. Chem. Soc. 77:2182(1955).

60. Mozingo, R., Org. Syn. 26:77(1946).

61. Millard, E. C., Schroeder, L. R., and Thompson, N. S., Carbohyd. Res. 56:259(1977).

62. Ballou, C. E., J. Am. Chem. Soc. 79:165(1957).

63. Ballou, C. E., Roseman, S., and Link, K. P., J. Am. Chem. Soc. 73:1140(1951).

64. Rosenthal, S. N. and Fendler, J. H., Adv. in Phys. Org. Chem. 9:279(1974).

65. Buchanan, J. G., Clode, D. M., and Vethaviyasar, N., J. Chem. Soc. Perkin I: 1149(1976).

66. Perlin, A. S., Int. Rev. Sci.: Org. Chem. Ser. Two. 7:1(1976).

67. Cohen, S., Levey, D., and Bergmann, E. D., Chem. Ind. (London) 43:1802(1964).

68. Garegg, P. J., Acta Chem. Scand. 14:957(1960).

69. Schroeder, L. R., Counts, K. M., and Haigh, F. C., Carbohyd. Res. 37:368(1974).

70. Hanessian, S. and Banoub, J., Carbohyd. Res. 53:C13-16(1977).

71. Whistler, R. L., Bachrach, J., and Tu, C. C., J. Am. Chem. Soc. 74:3059(1952).

72. Hultmen, D. P., Schroeder, L. R., and Haigh, F. C., J. Chem. Soc. Perkin II: 1525(1972).

73. Bywater, R. A. S., Haworth, W. N., Hirst, E. L., and Peat, S., J. Chem. Soc. 1937:1983.

74. Millard, E. C., The degradation of selected 1,5-anhydroalditols by molecular oxygen in alkaline media. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1976.

75. Petersson, G., Tetrahedron 26:3413(1970).

76. Machell, G. and Richards, G. N., J. Chem. Soc. 1960:1924.

77. Malinen, R. and Sjostrom, E., Papper Och Tra 8:451(1972).

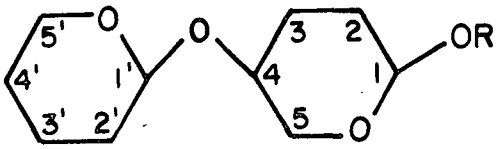
78. Johnsen, B. and Sorensen, P. E., Acta Chem. Scand. A 33:241(1979).

79. Marchessault, R. H., and Timmell, T. E., J. Polymer Sci. Part C 2:49(1963).

APPENDIX I

^{13}C-NMR SPECTRA

This appendix contains the ^{13}C-NMR spectra of all the compounds shown in Fig. 5 and also the spectra of the derivatives used for characterization of certain compounds. Each spectrum contains a tabular list of chemical shift values that are assigned to the various carbon atoms in the compound. The ring carbons in the disaccharide molecules are numbered as shown below.



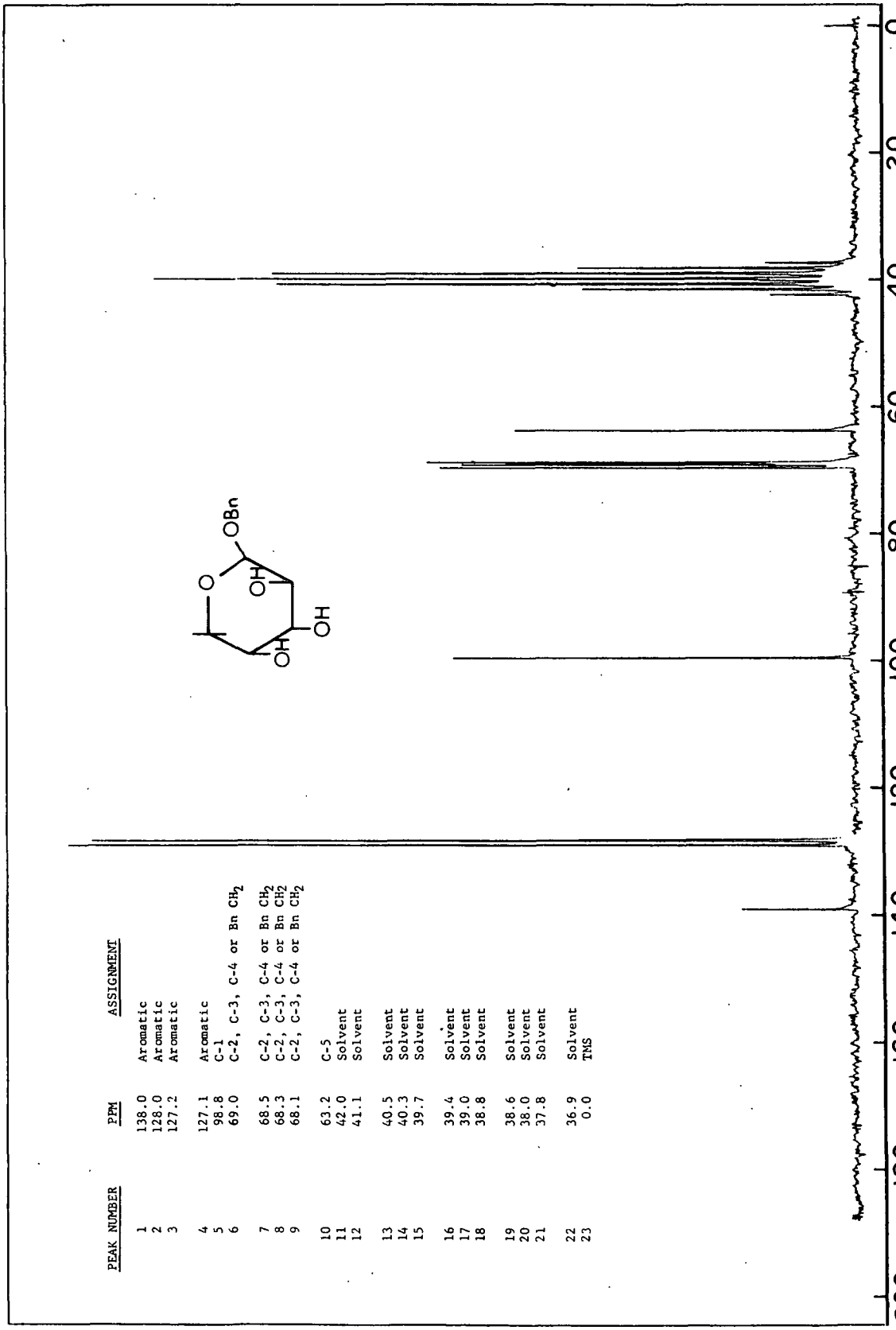
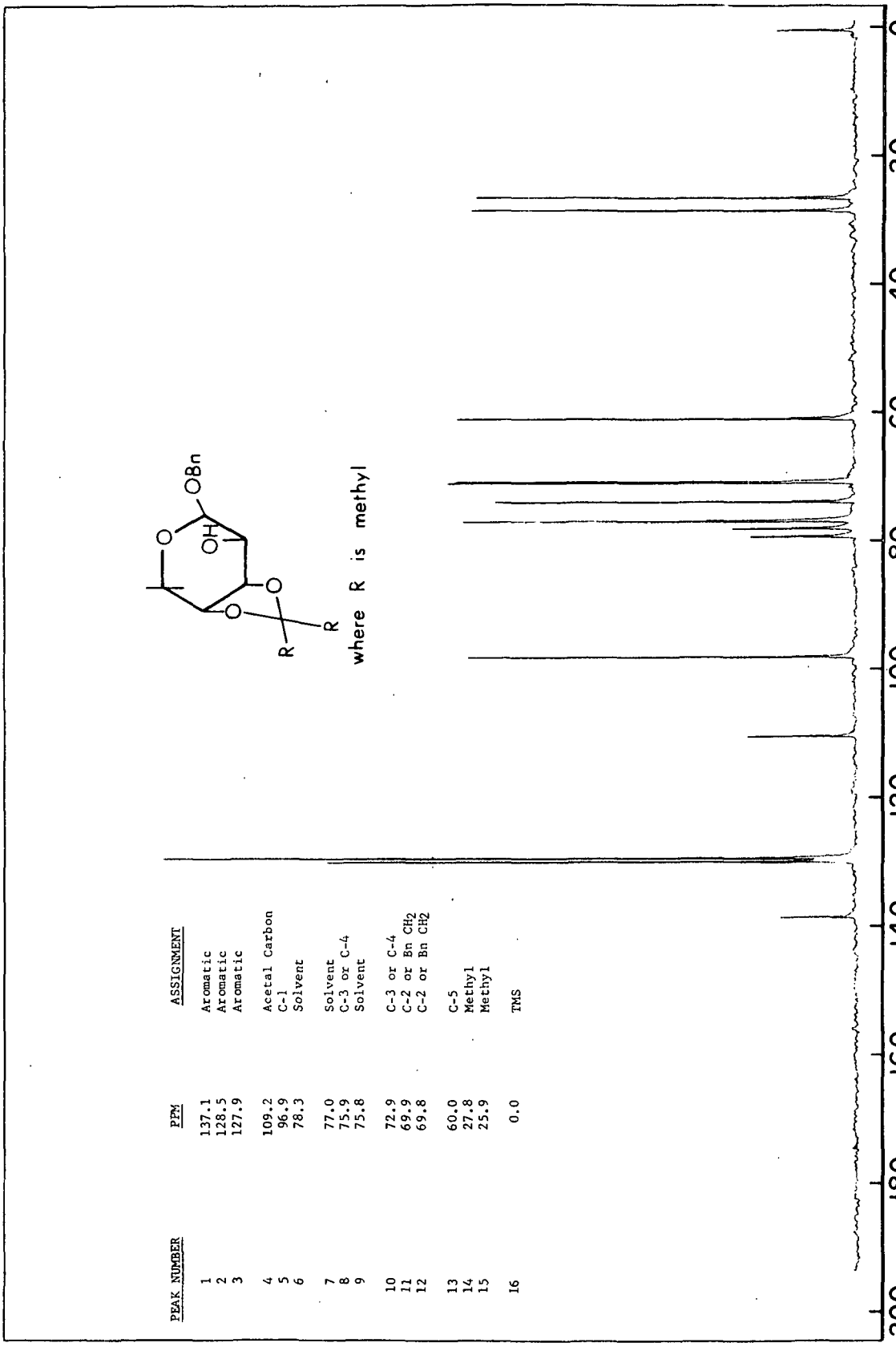


Figure 20. The ^{13}C-NMR Spectrum of Benzyl β-D-Arabinopyranoside in DMSO-d_6



PEAK NUMBER	PPM	ASSIGNMENT
1	137.1	Aromatic
2	128.5	Aromatic
3	127.9	Aromatic
4	109.2	Acetal Carbon
5	96.9	C-1
6	78.3	Solvent
7	77.0	Solvent
8	75.9	C-3 or C-4
9	75.8	Solvent
10	72.9	C-3 or C-4
11	69.9	C-2 or Bn CH_2
12	69.8	C-2 or Bn CH_2
13	60.0	C-5
14	27.8	Methyl
15	25.9	Methyl
16	0.0	TMS

Figure 21. The ^{13}C-NMR Spectrum of Benzyl 3,4-O-Isopropylidene-β-D-Arabinopyranoside in Chloroform-d

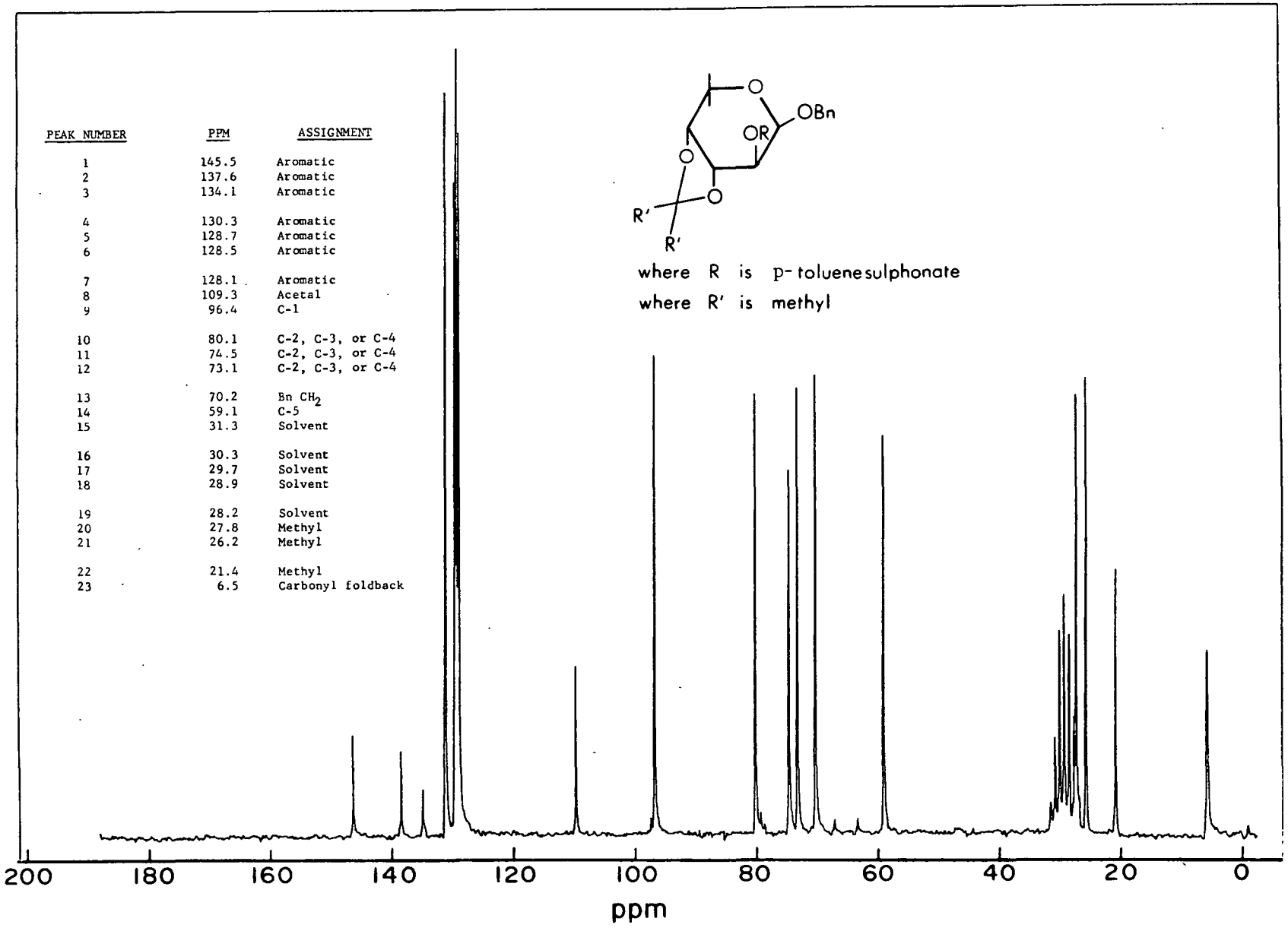
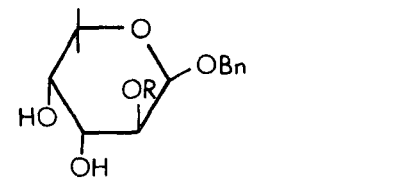


Figure 22. The ^{13}C-NMR Spectrum of Benzyl 3,4-O-Isopropylidene-2-O-p-Toluenesulfonyl-β-D-Arabinopyranoside in Acetone-d_6

PEAK NUMBER	PPM	ASSIGNMENT
1	145.2	Aromatic
2	129.8	Aromatic
3	129.6	Aromatic
4	128.4	Aromatic
5	128.3	Aromatic
6	127.9	Aromatic
7	127.8	Aromatic
8	95.8	C-1
9	78.2	C-2
10	78.1	Solvent
11	77.0	Solvent
12	75.7	Solvent
13	69.6	C-3, C-4 or Bn CH_2
14	69.4	C-3, C-4 or Bn CH_2
15	67.2	C-3, C-4 or Bn CH_2
16	62.0	C-5
17	21.6	Methyl
18	0.0	TMS



where R is p-toluenesulphonate

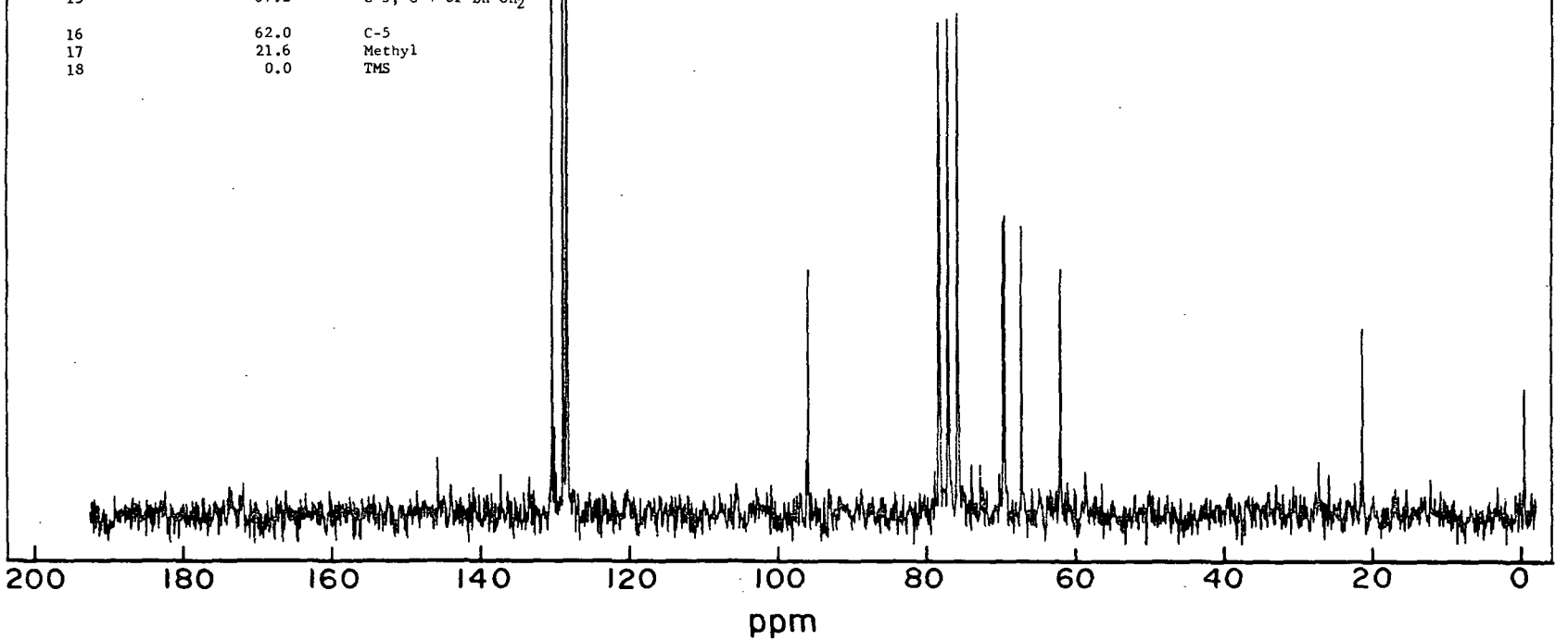


Figure 23. The ^{13}C-NMR Spectrum of Benzyl 2-O-p-Toluenesulfonyl-β-D-Arabinopyranoside in Chloroform-d

PEAK NUMBER	PPM	ASSIGNMENT
1	137.0	Aromatic
2	128.4	Aromatic
3	128.0	Aromatic
4	127.9	Aromatic
5	93.6	C-1
6	78.4	Solvent
7	77.1	Solvent
8	75.8	Solvent
9	70.0	Bn CH_2
10	62.1	C-5
11	61.7	C-4
12	51.9	C-2 and C-3
13	0.0	TMS

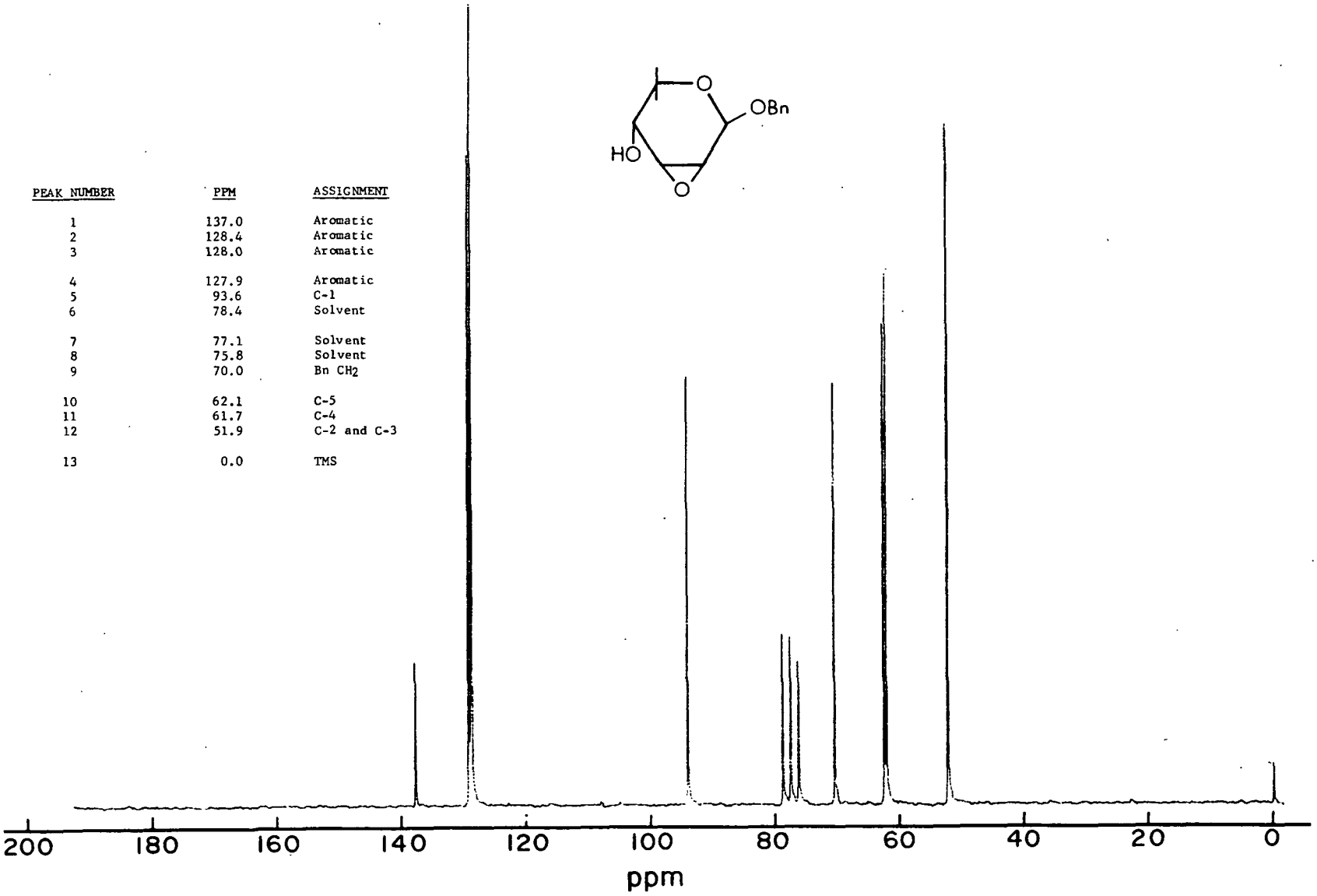


Figure 24. The ^{13}C-NMR Spectrum of Benzyl 2,3-Anhydro-β-D-Ribopyranoside in Chloroform-d

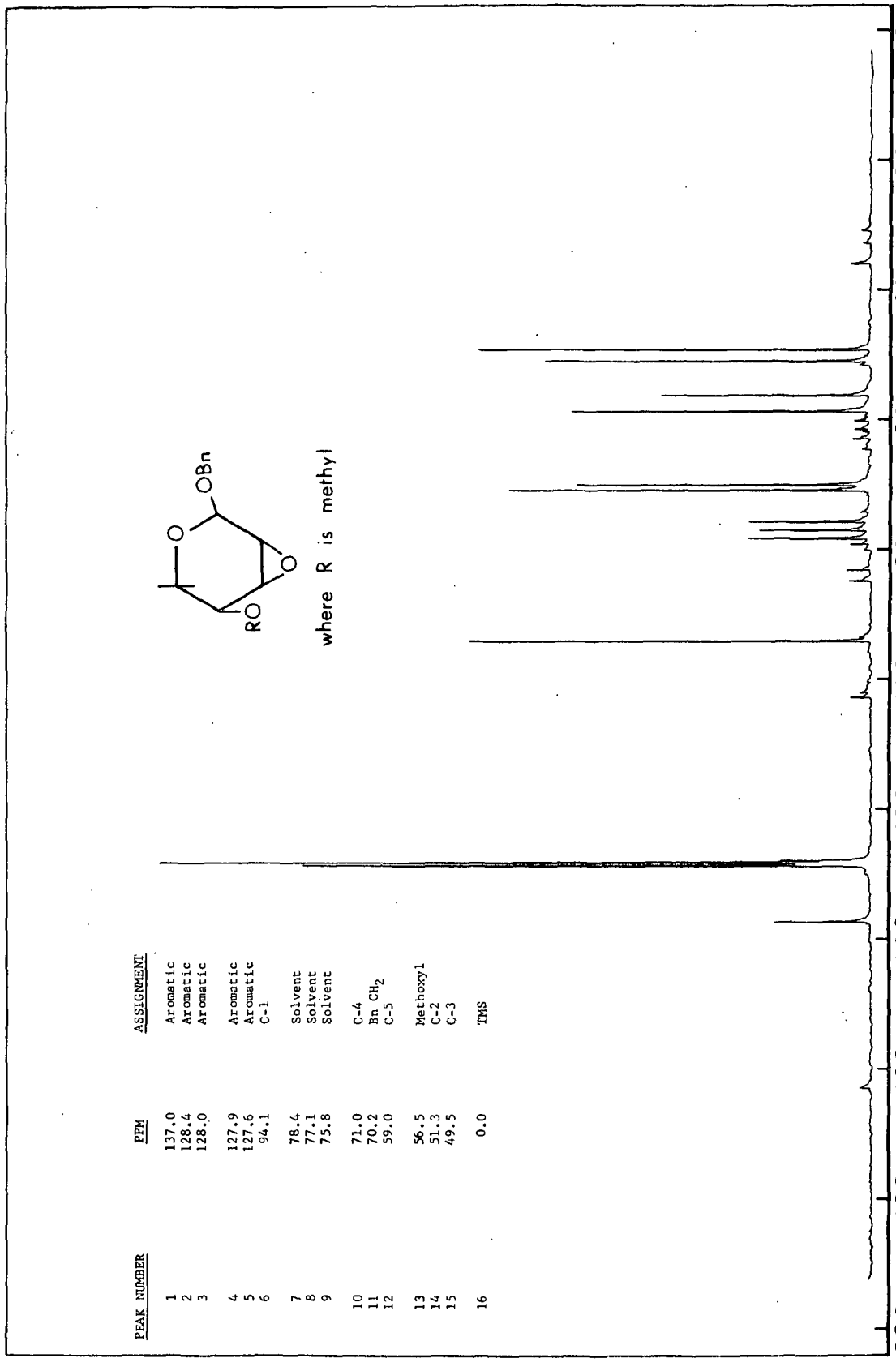


Figure 25. The ^{13}C-NMR Spectrum of Benzyl 2,3-Anhydro-4-O-Methyl-β-D-Ribopyranoside in Chloroform-d

PEAK NUMBER	PPM	ASSIGNMENT
1	137.4	Aromatic
2	128.1	Aromatic
3	127.6	Aromatic
4	127.5	Aromatic
5	103.5	C-1'
6	94.2	C-1
7	76.5	C-3'
8	72.9	C-2'
9	69.8	C-4' or C-4
10	69.3	Bn CH_2 and C-4' or C-4
11	65.6	C-5'
12	58.8	C-5
13	52.0	C-3
14	51.1	C-2
15	41.9	Solvent
16	41.1	Solvent
17	40.3	Solvent
18	39.4	Solvent
19	38.5	Solvent
20	37.7	Solvent
21	36.9	Solvent
22	0.0	TMS

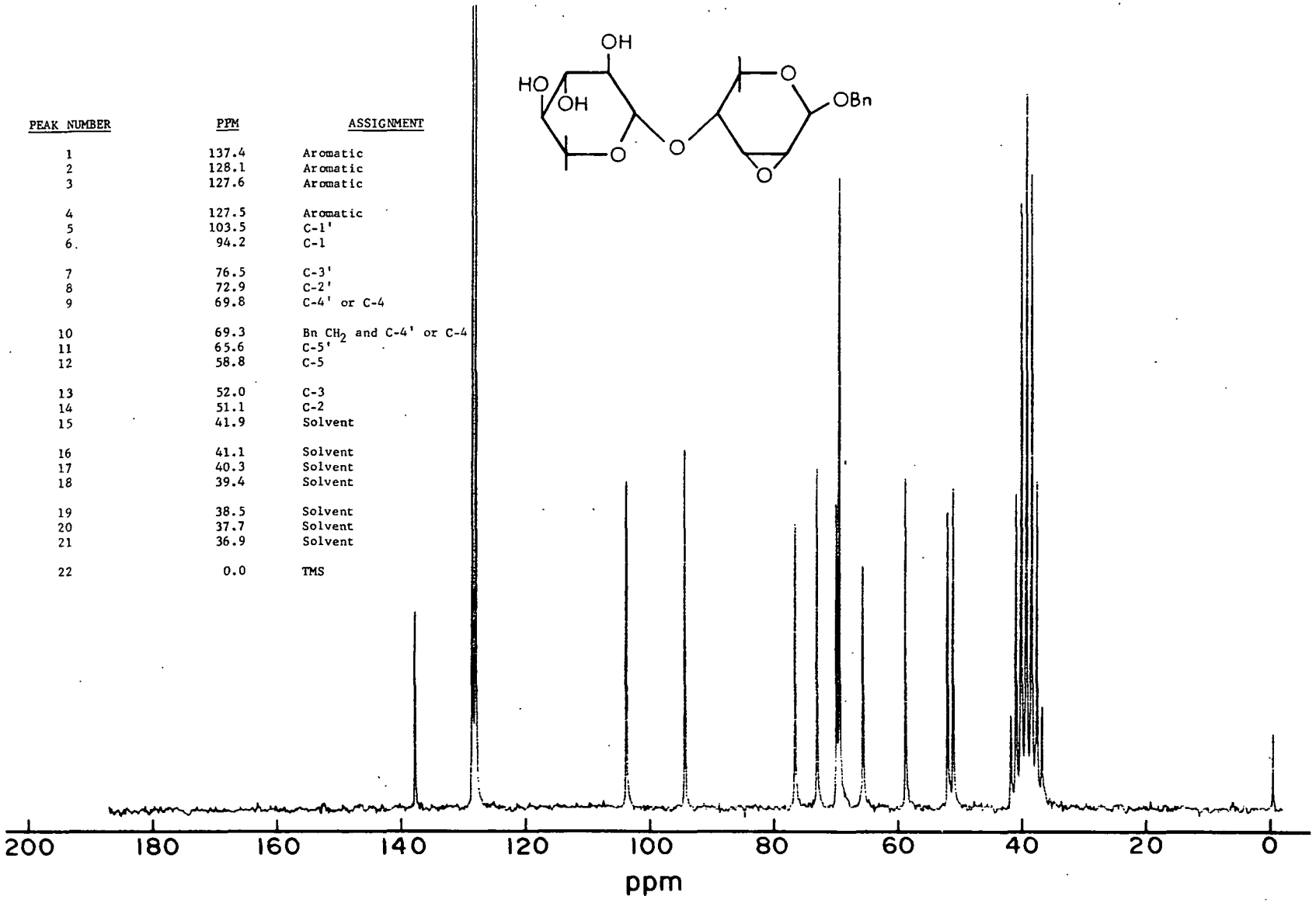


Figure 26. The ^{13}C-NMR Spectrum of Benzyl 2,3-Anhydro-4-O-(β-D-Xylopyranosyl)-β-D-Ribopyranoside (XXV) in DMSO-d_6

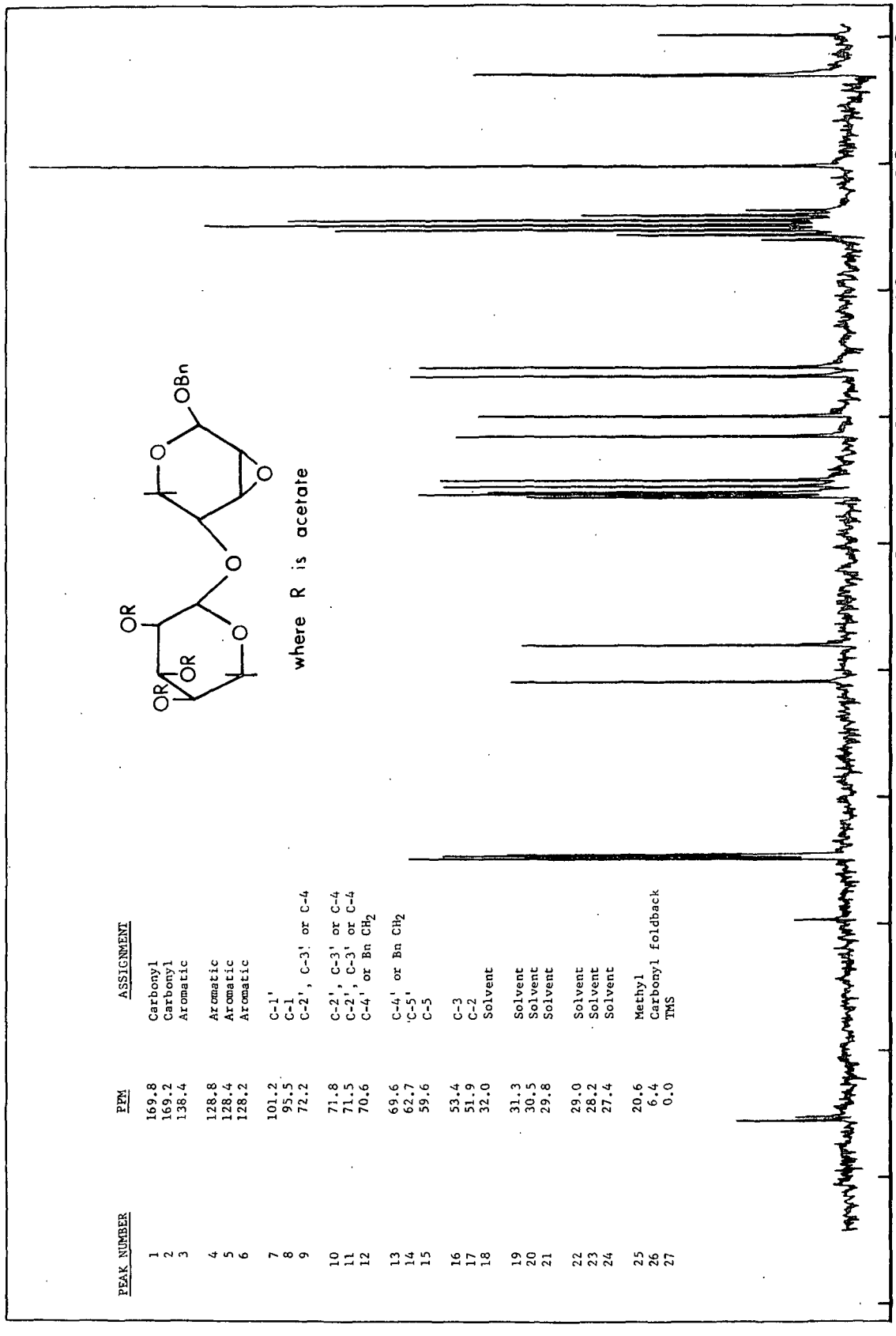


Figure 27. The ^{13}C-NMR Spectrum of Benzyl 2,3-Anhydro-4-O-(2',3',4'-Tri-O-Acetyl-β-D-Xylopyranosyl)-β-D-Ribopyranoside in Aceton-d_6

PEAK NUMBER	PPM	ASSIGNMENT
1	137.8	Aromatic
2	128.0	Aromatic
3	127.4	Aromatic
4	102.7	C-1'
5	101.8	C-1
6	76.1	C-3' or C-4
7	75.3	C-3' or C-4
8	74.1	C-2, C-2' or C-3
9	73.1	C-2, C-2' or C-3
10	72.4	C-2, C-2' or C-3
11	69.6	Bn CH_2
12	69.4	C-4'
13	65.7	C-5'
14	63.1	C-5
15	56.0	
16	41.8	Solvent
17	41.0	Solvent
18	40.2	Solvent
19	39.3	Solvent
20	38.4	Solvent
21	37.6	Solvent
22	36.8	Solvent
23	18.4	
24	0.0	TMS

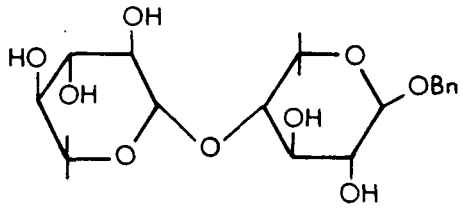


Figure 28. The ^{13}C-NMR Spectrum of Benzyl β-Xylobioside (XXVI) in DMSO-d_6

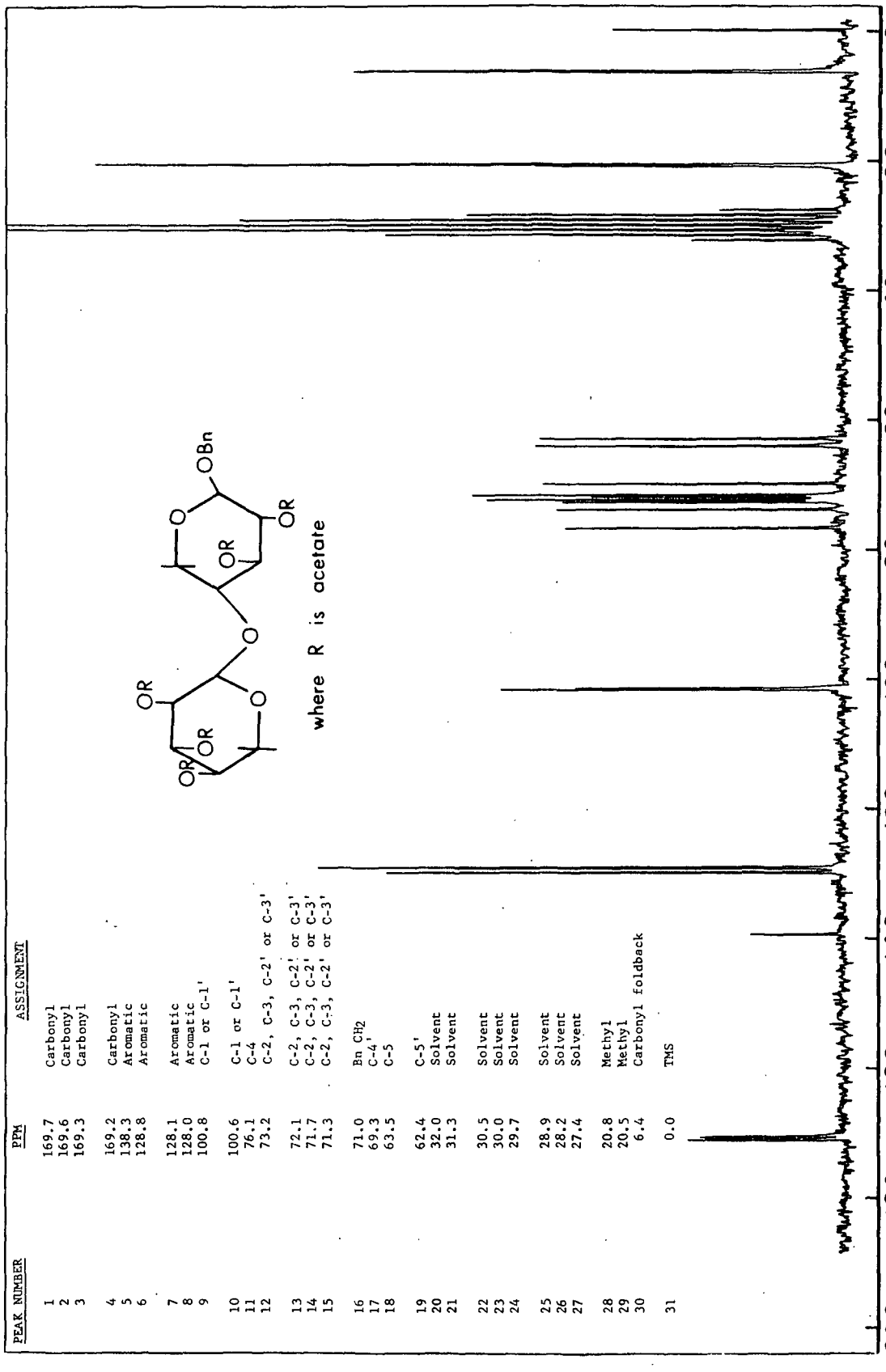


Figure 29. The ^{13}C-NMR Spectrum of Benzyl β-Xylobioside Pentaacetate in Acetone-d_6

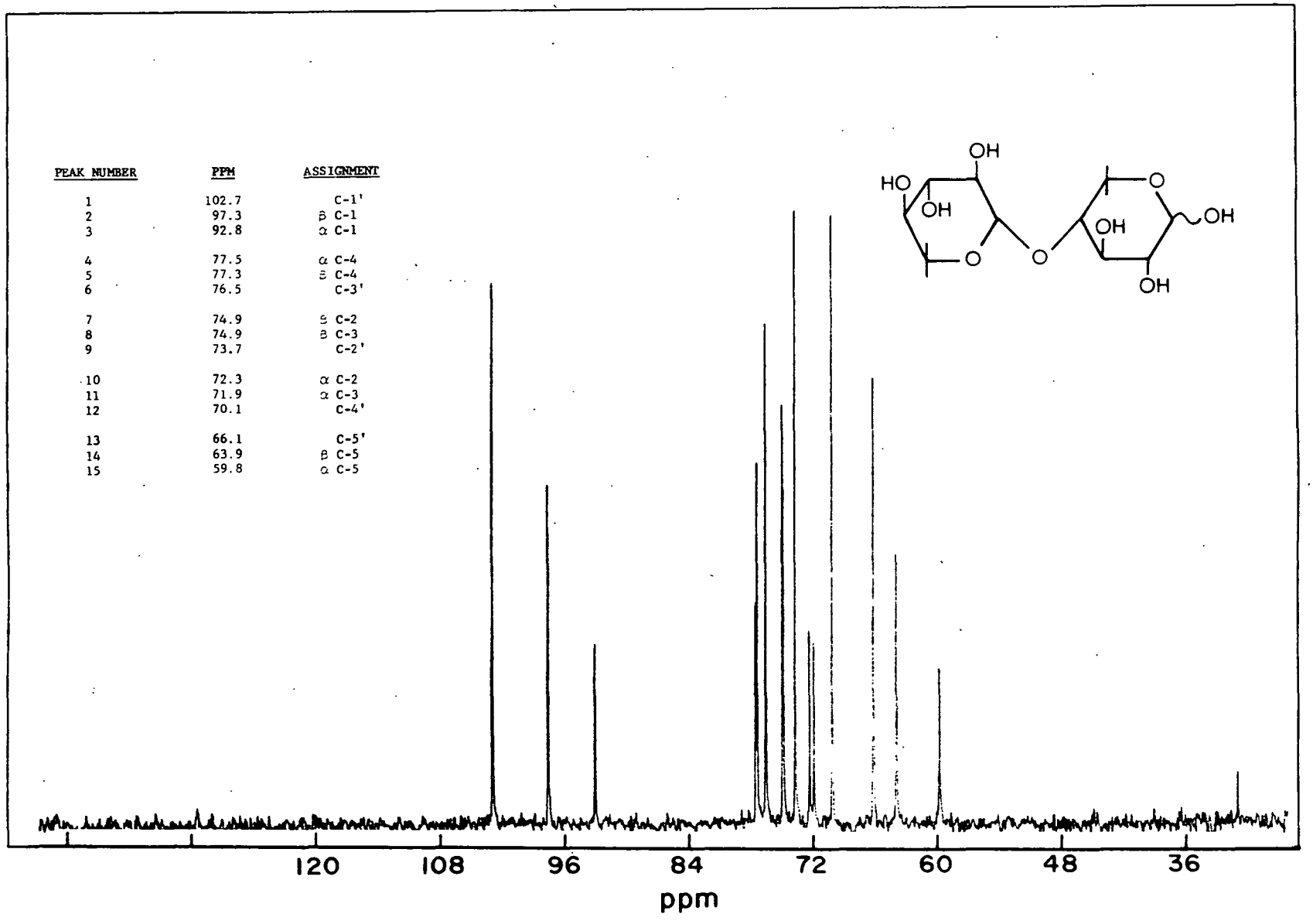
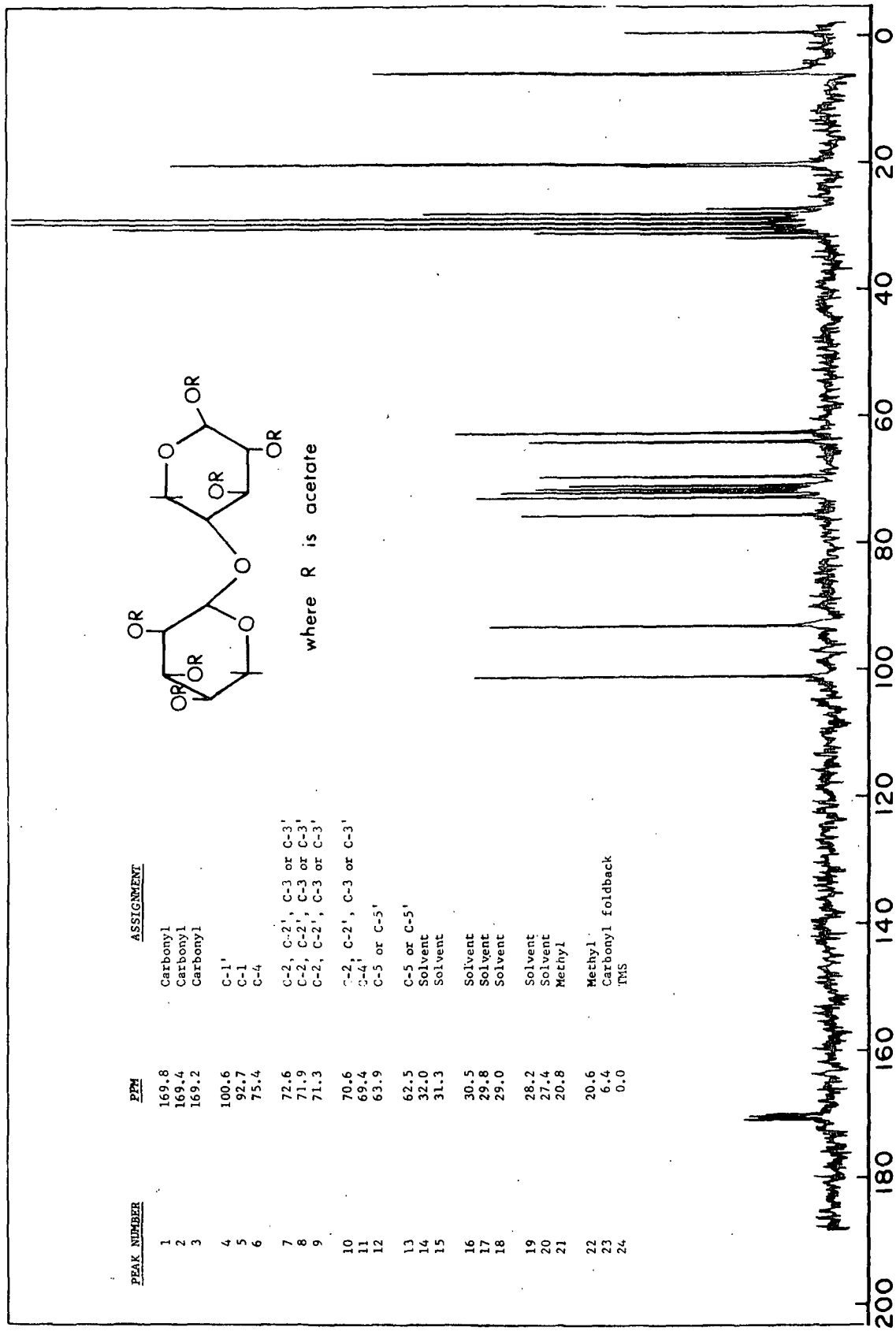


Figure 7. The ^{13}C-NMR Spectrum of Xylobiose in D_2O



PEAK NUMBER	PPM	ASSIGNMENT
1	169.8	Carbonyl
2	169.4	Carbonyl
3	169.2	Carbonyl
4	100.6	C-1'
5	92.7	C-1
6	75.4	C-4
7	72.6	C-2, C-2', C-3 or C-3'
8	71.9	C-2, C-2', C-3 or C-3'
9	71.3	C-2, C-2', C-3 or C-3'
10	70.6	C-2, C-2', C-3 or C-3'
11	69.4	C-4'
12	63.9	C-5 or C-5'
13	62.5	C-5 or C-5'
14	32.0	Solvent
15	31.3	Solvent
16	30.5	Solvent
17	29.8	Solvent
18	29.0	Solvent
19	28.2	Solvent
20	27.4	Solvent
21	20.8	Methyl
22	20.6	Methyl
23	6.4	Carbonyl foldback
24	0.0	TMS

Figure 6. The ^{13}C-NMR Spectrum of β-Xylobiose Hexaacetate in Acetone -d_6

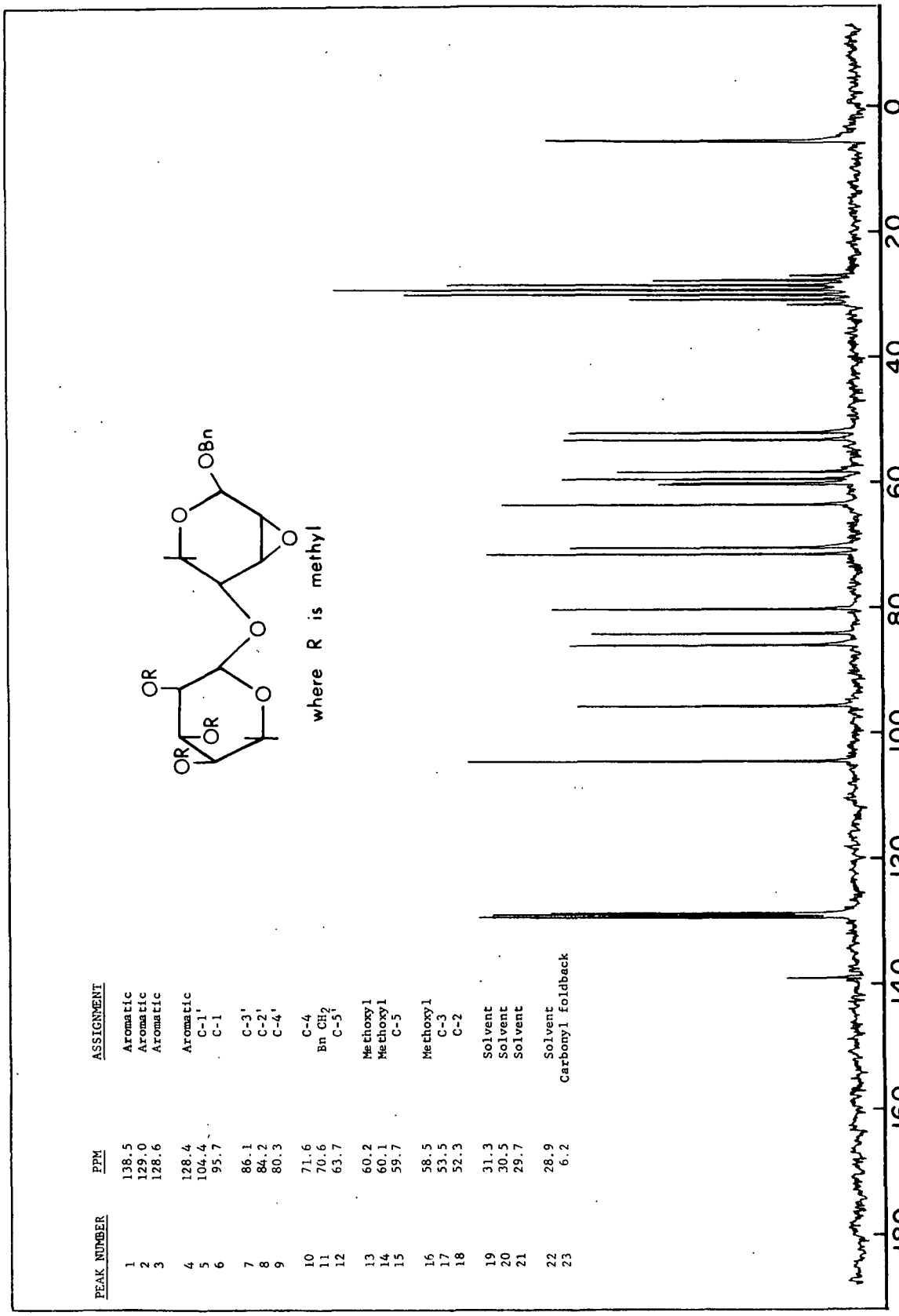
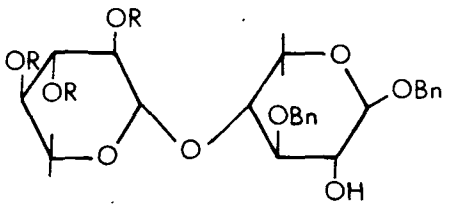


Figure 30. The ^{13}C-NMR Spectrum of Benzyl 2,3-Anhydro-4-O-(2',3',4'-Tri-O-Methyl-β-D-Xylopyranosyl)-β-D-Ribopyranoside (XXVII) in Acetone-d_6

PEAK NUMBER	PPM	ASSIGNMENT
1	140.0	Aromatic
2	138.6	Aromatic
3	128.8	Aromatic
4	128.5	Aromatic
5	128.2	Aromatic
6	128.0	Aromatic
7	127.8	Aromatic
8	127.4	Aromatic
9	103.3	C-1'
10	102.6	C-1
11	85.9	C-3'
12	84.2	C-2'
13	82.4	C-3
14	80.1	C-4'
15	76.7	C-4
16	74.3	Bn CH_2
17	73.6	C-2
18	70.6	Bn CH_2
19	63.6	C-5 or C-5'
20	63.1	C-5 or C-5'
21	60.5	Methoxyl
22	60.4	Methoxyl
23	58.4	Methoxyl
24	31.3	Solvent
25	30.5	Solvent
26	29.7	Solvent
27	28.9	Solvent
28	28.2	Solvent
29	6.4	Carbonyl foldback
30	0.0	TMS



where R is methyl

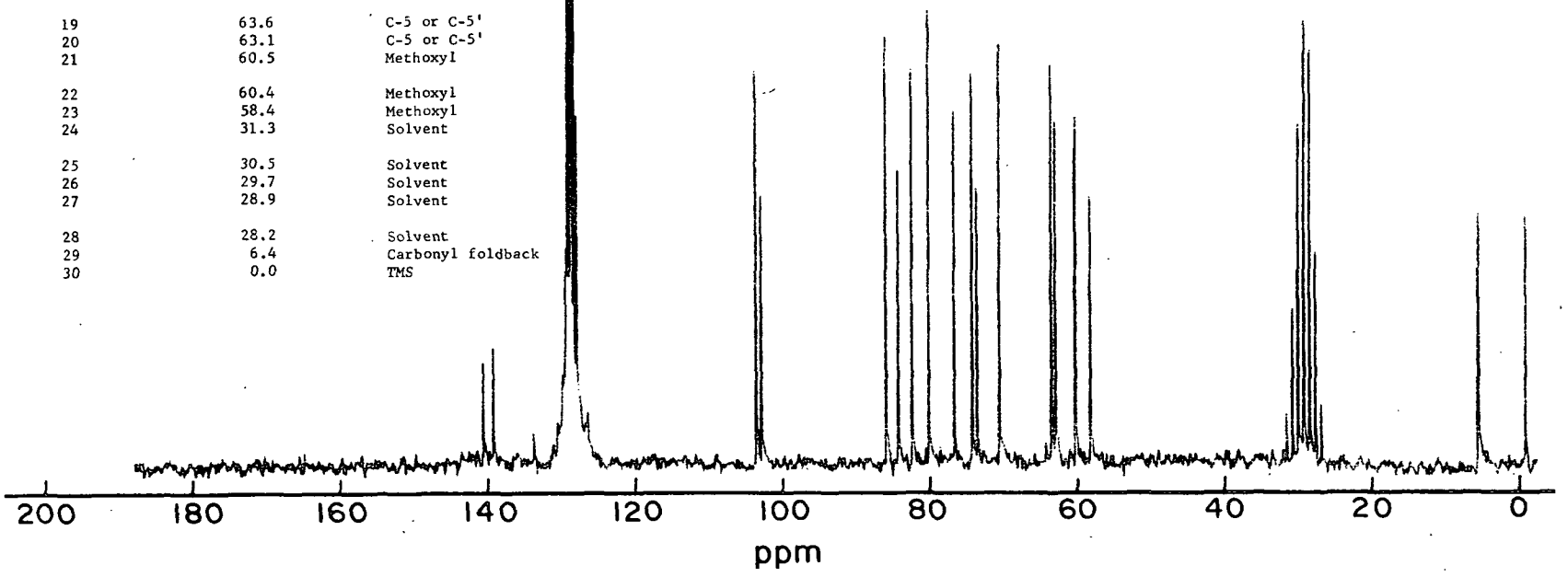


Figure 31. The ^{13}C-NMR Spectrum of Benzyl 3-O-Benzyl-4-O-(2',3',4'-Tri-O-Methyl-β-D-Xylopyranosyl)-β-D-Xylopyranoside in Acetone-d_6

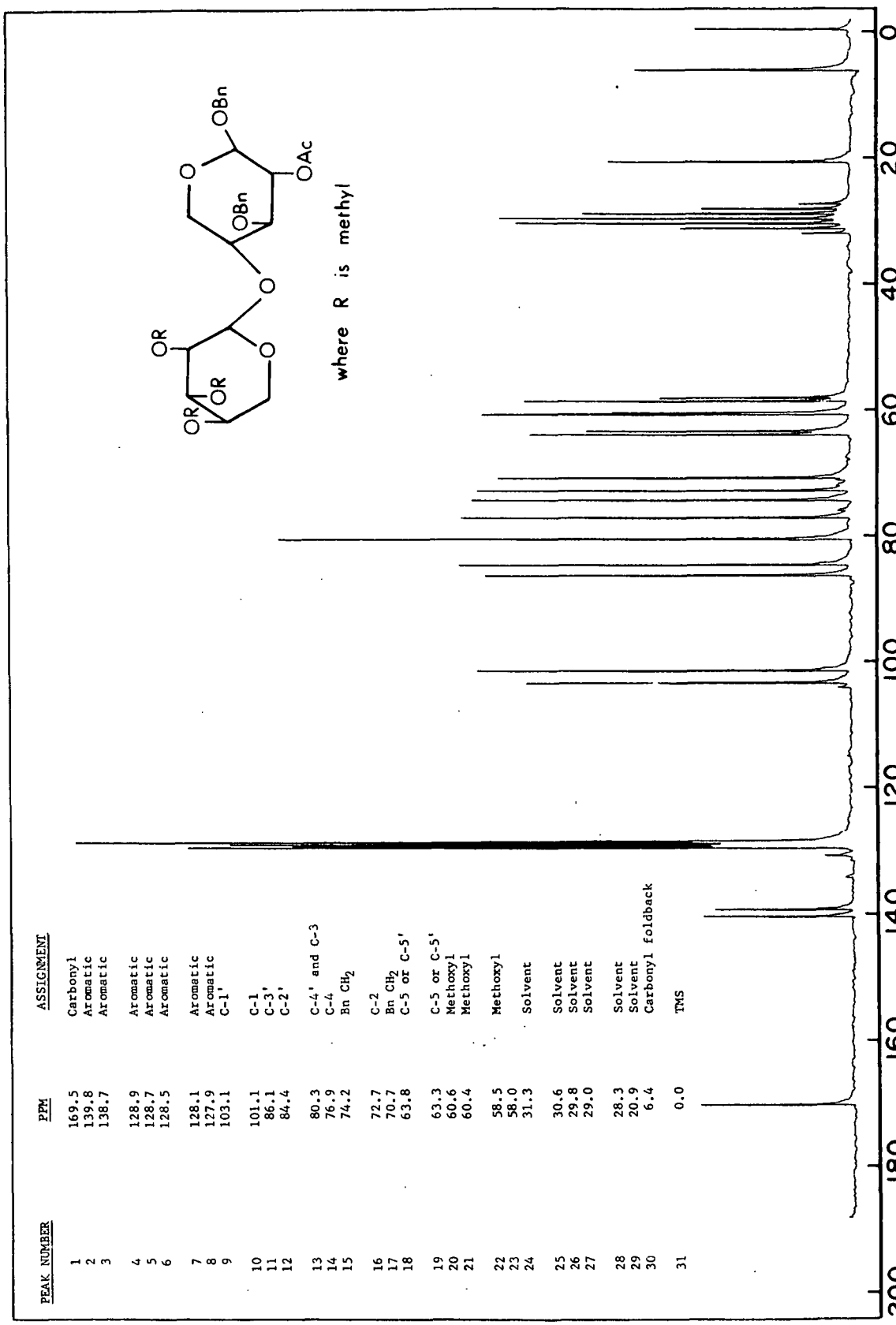


Figure 32. The ^{13}C-NMR Spectrum of Benzyl 2-O-Acetyl-3-O-Benzyl-4-O-(2',3',4'-Tri-O-Methyl-β-D-Xylopyranosyl)-β-D-Xylopyranoside in Acetone-d_6

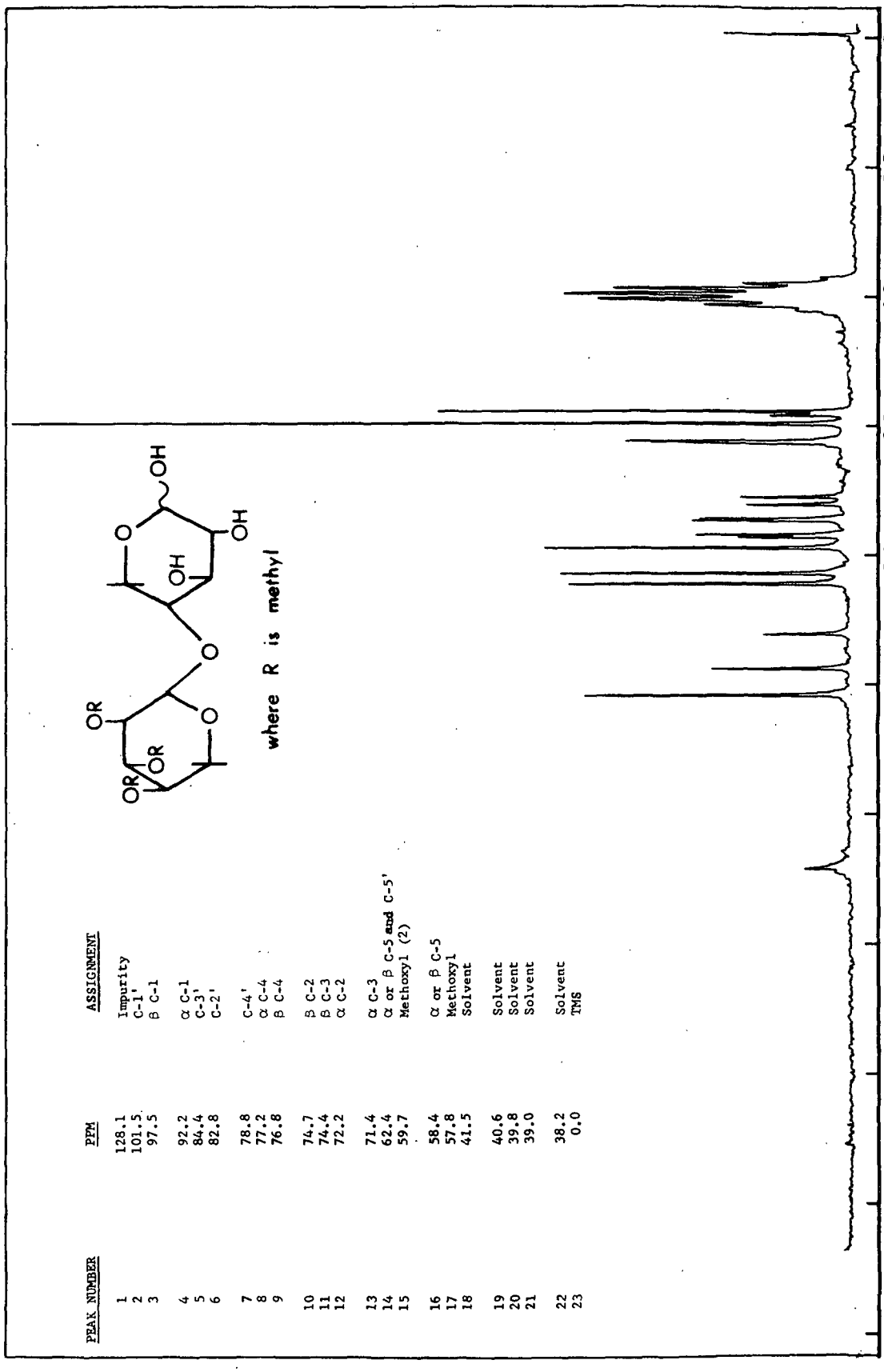


Figure 8. The ^{13}C-NMR Spectrum of 2',3',4'-Tri-O-Methyl-Xylobiose (XXII) in DMSO-d_6

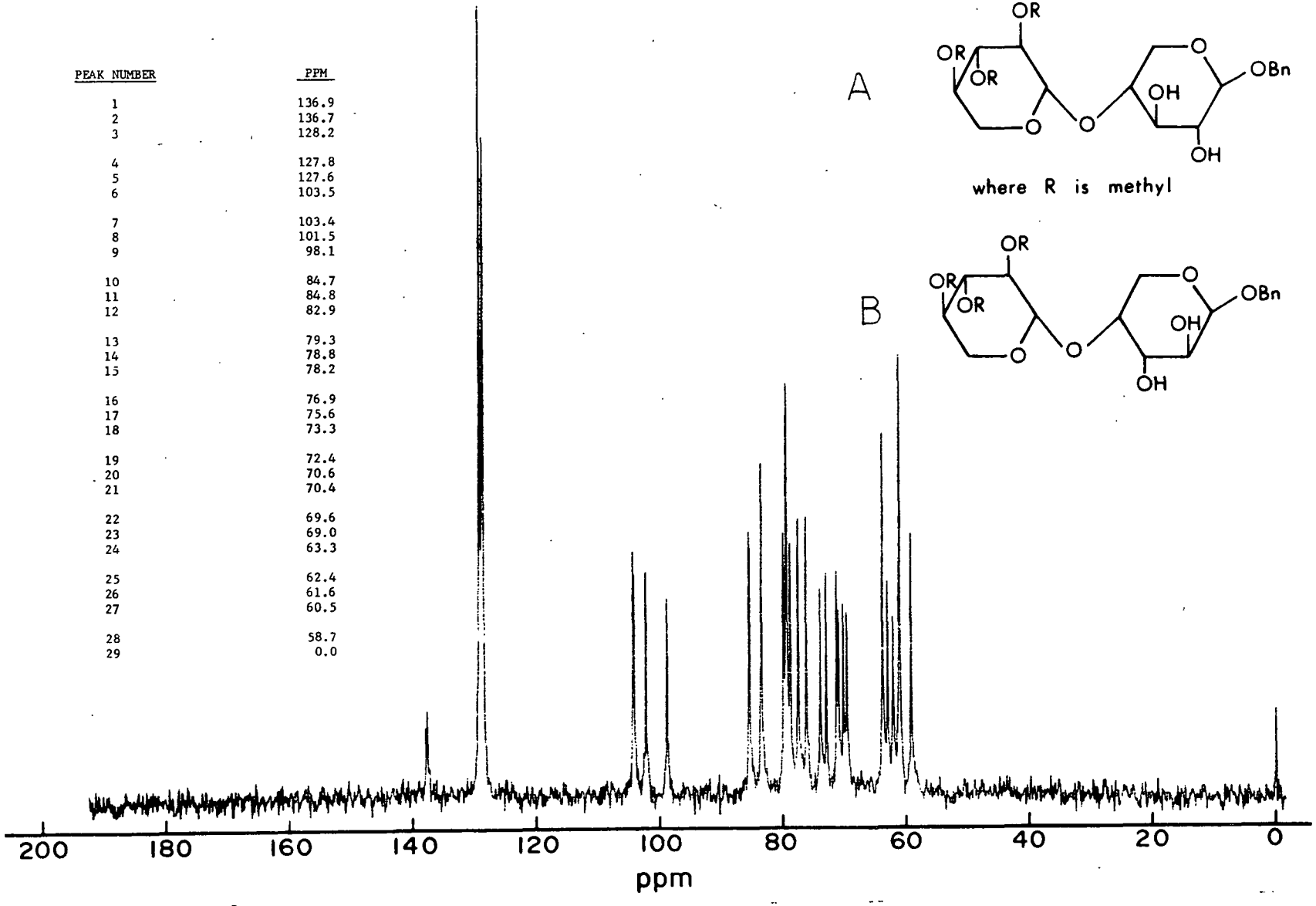


Figure 9. The ^{13}C-NMR Spectrum of the Product (A and B) From the Reaction of Benzyl 2,3-anhydro-4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-ribopyranoside and Sodium Hydroxide Obtained in Chloroform-d: A, Benzyl 4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-xylopyranoside; B, Benzyl 4-O-(2',3',4'-tri-O-methyl-β-D-xylopyranosyl)-β-D-arabinopyranoside

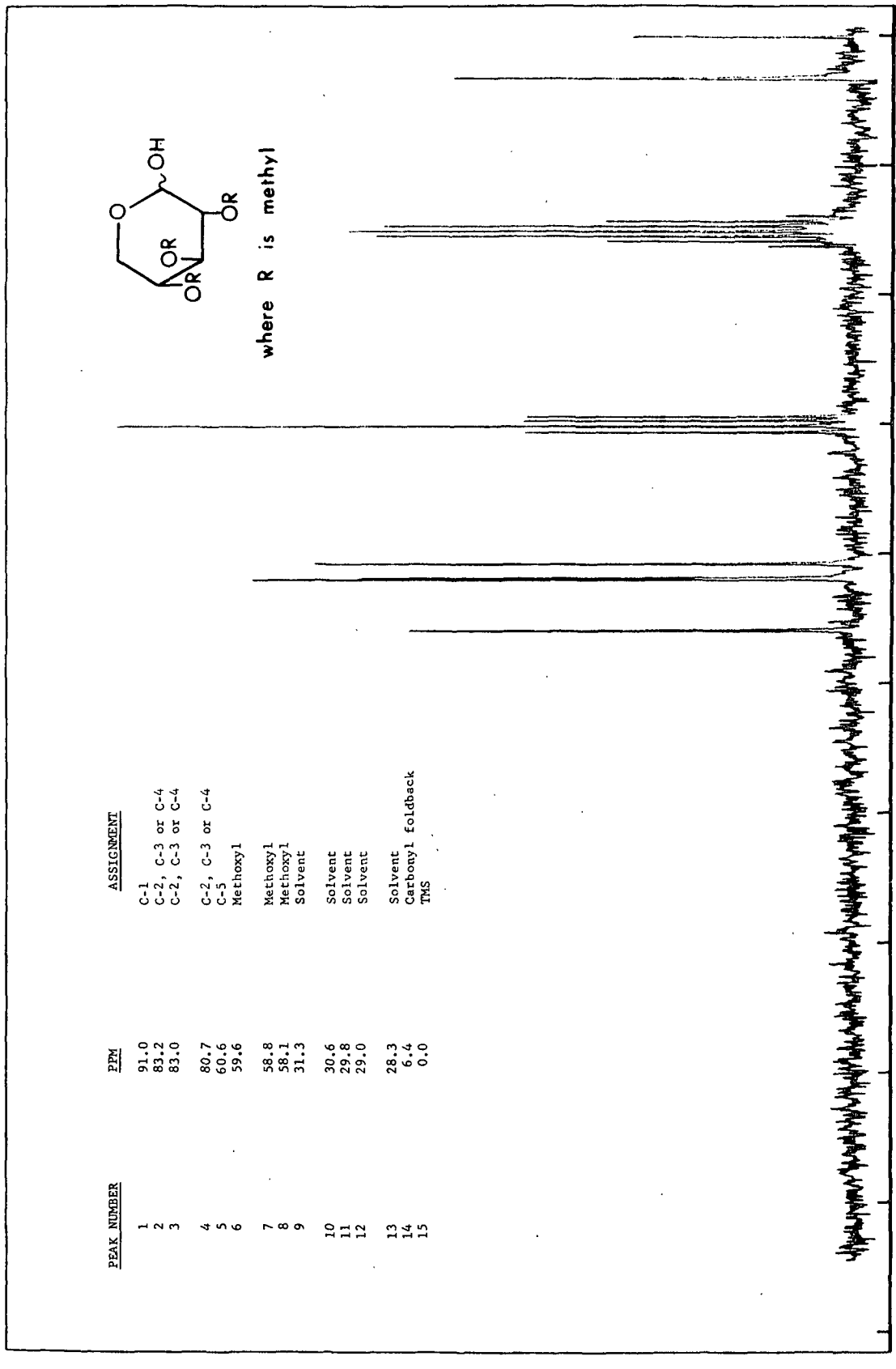


Figure 33. The ^{13}C-NMR Spectrum of 2,3,4-Tri-O-Methyl-D-Xylose in Acetone-d_6

APPENDIX II

KINETIC DATA

This appendix contains the kinetic data that were used to calculate the pseudo-first-order rate constants (k_r) of degradation for xylobiose and 2',3',4'-tri-O-methyl-xylobiose.

TABLE VII

DEGRADATION OF XYLOBIOSE (0.0015M) IN 0.1N SODIUM HYDROXIDE AT 30°C

Time, min	X2, 10^3M
0	1.47
45	1.39
90	1.23
135	1.06
180	0.94
225	0.82
270	0.69
315	0.63
360	0.54

TABLE VIII

DEGRADATION OF XYLOBIOSE (0.0015M) IN 0.1N SODIUM HYDROXIDE WITH 2.4M SODIUM ACETATE AT 30°C

Time, min	X2, 10^3M
0	1.51
30	1.36
60	1.28
90	1.05
135	0.87
180	0.73
225	0.65
270	0.56

TABLE IX

DEGRADATION OF XYLOBIOSE (0.0015M) IN 2.5N SODIUM HYDROXIDE AT 30°C

Time, min	X2, 10^3M
0	1.51
20	1.40
40	1.35
60	1.18
80	1.13
100	1.05
120	0.95
150	0.83
180	0.74
210	0.64

TABLE X

DEGRADATION OF XYLOBIOSE (0.0015M) IN 2.5N SODIUM HYDROXIDE AT 30°C

Time, min	X2, 10^3M
0	1.48
40	1.30
80	1.15
100	1.05
120	0.97
150	0.81
180	0.68
210	0.66

TABLE XI

DEGRADATION OF 2',3',4'-TRI-O-METHYL XYLOBIOSE (0.0012M) IN 0.1N SODIUM HYDROXIDE AT 30°C

Time, min	TMX2, 10^3M
0	1.20
45	1.10
90	0.98
135	0.88
180	0.73
225	0.68
270	0.62
315	0.51
360	0.47
406	0.41

TABLE XII

DEGRADATION OF 2',3',4'-TRI-O-METHYLXYLOBIOSE (0.0012M) IN 0.1N SODIUM HYDROXIDE WITH 2.4M SODIUM ACETATE AT 30°C

Time, min	TMX2, 10^3M
0	1.20
20	1.16
60	1.02
90	0.94
120	0.82
180	0.67
240	0.57
300	0.48

TABLE XIII

DEGRADATION OF 2',3',4'-TRI-O-METHYL XYLOBIOSE (0.0012M) IN 2.5N SODIUM HYDROXIDE AT 30°C

Time, min	TMX2, 10^3M
0	1.22
20	1.02
40	1.07
60	1.02
90	0.86
120	0.77
180	0.65
300	0.37

TABLE XIV

DEGRADATION OF 2',3',4'-TRI-O-METHYL XYLOBIOSE (0.0012M) IN 2.5N SODIUM HYDROXIDE AT 30°C

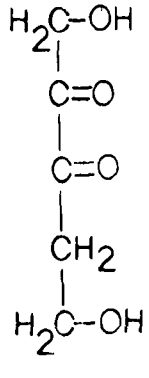
Time, min	TMX2, 10^3M
0	1.25
20	1.18
40	1.04
120	0.78
150	0.68
180	0.61
240	0.45
300	0.39

APPENDIX III

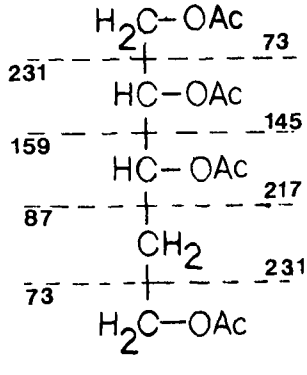
4-DEOXY-2,3-PENTODIULOSE: A REACTIVE INTERMEDIATE

An additional product was observed in the kinetic chromatographic analyses of degradation of both disaccharides in 0.1M sodium hydroxide. Since only neutral products were analyzed by this workup procedure, the product was thought to be the reduced, acetylated derivative of 4-deoxy-2,3-pentodiulose (XXX), i.e., the acetylated 2-deoxypentitol (XL). In support of this assumption, the observed product has the same GLC retention time and mass spectrum as the acetylated alditol derived from 2-deoxy-ribose.

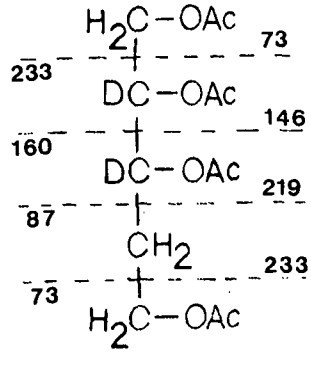
The mass spectrum of XXX, as the alditol peracetate (XL) (Fig. 34, A), is consistent with the 2-deoxypentitol structure. The mass spectrum of XL is characterized by the presence of an M-59 peak at m/e 245 which is typical of acetate derivatives. The remaining fragments useful in identification result from carbon-carbon bond cleavage. Strong peaks are present at m/e 159 and m/e 145 which result from C-2 - C-3 bond cleavage. A weak, but important peak is observed at m/e 217 that was caused by cleavage of the C-3 - C-4 bond.



XXX



XL



XLI

The number of carbonyl groups present in the original, unreduced product could not be determined from the mass spectrum of XL alone. However, a technique employing reduction of the unknown with sodium borodeuteride (vs. sodium borohydride in previous samples) enabled the determination of the number and position of carbonyl groups. Basically, this technique reduces the unknown to a 2-deoxypentitol with deuterium atoms incorporated at carbon atoms that had carbonyl functions in the original compound. Therefore, comparison of the mass spectra of the alditol acetates resulting from reduction with sodium borohydride (Fig. 34, A) and sodium borodeuteride (Fig. 34, B) enable determination of the number and position of carbonyl functions in the product.

As expected, the mass spectrum of XLI was very similar to XL except that several diagnostic peaks had increased by one or two mass units. For example, the M-59 peak had increased from m/e 245 to m/e 247 which indicates that the unknown has two carbonyl functions. Similarly, the larger fragment resulting from C-1 - C-2 bond cleavage increased two mass units to m/e 233 indicating both carbonyl groups were retained in that portion of the parent compound. The two fragments resulting from C-2 - C-3 bond cleavage were both increased by one mass unit to m/e 160 and m/e 146. The larger fragment resulting from C-3 - C-4 cleavage has also increased two mass units to m/e 219.

The information from the two mass spectra indicate that the unknown is 4-deoxy-2,3-pentodiulose, a reactive intermediate leading to reaction products associated with the reducing end unit of the disaccharides (37).

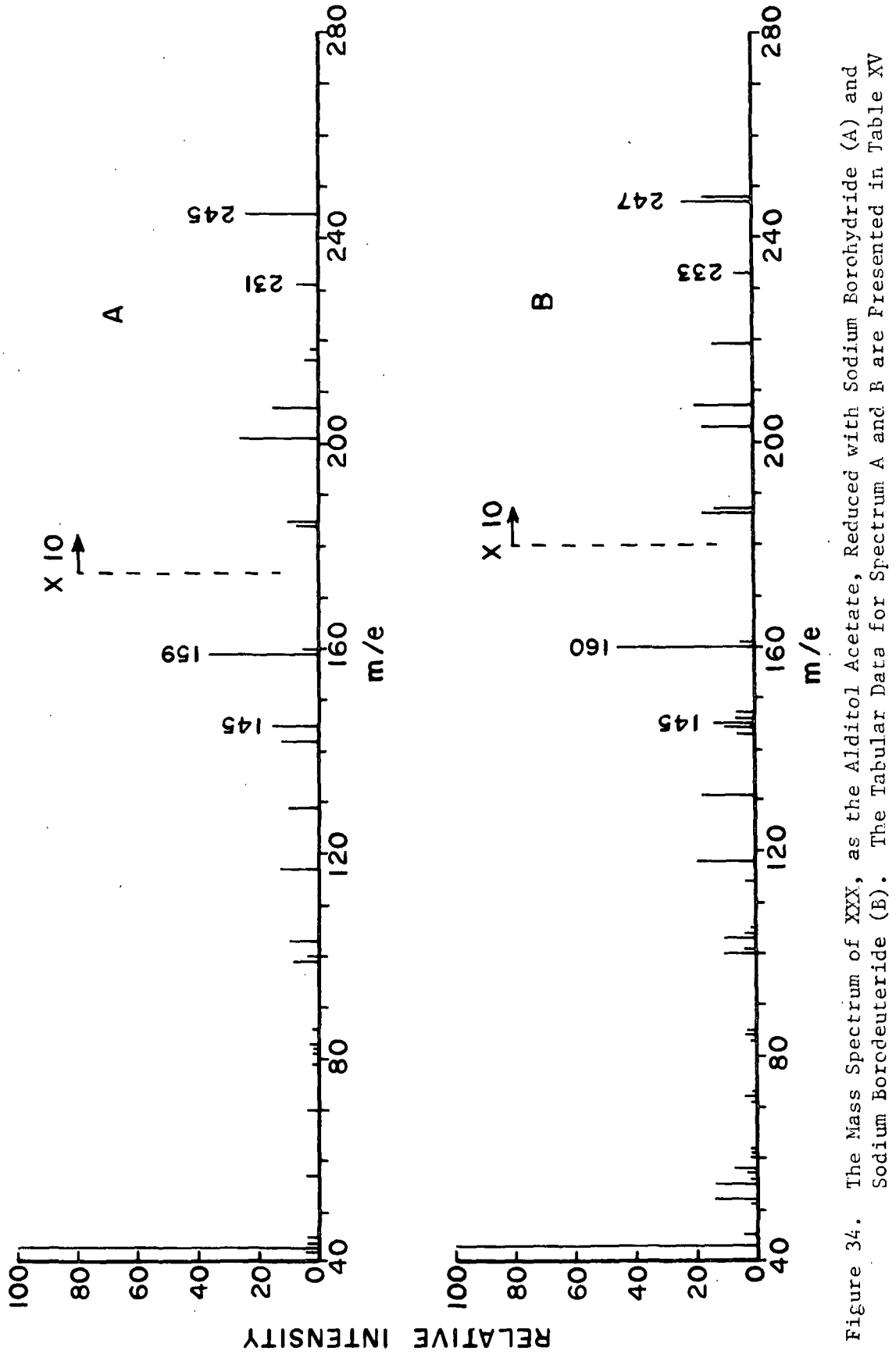


Figure 34. The Mass Spectrum of XXX, as the Alditol Acetate, Reduced with Sodium Borohydride (A) and Sodium Borodeuteride (B). The Tabular Data for Spectrum A and B are Presented in Table XV and XVI, Respectively

TABLE XV

MASS SPECTRAL DATA FOR 4-DEOXY-2,3-PENTODIULOSE REDUCED WITH SODIUM BOROHYDRIDE (ACETATE DERIVATIVE)[a,b]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
245	2.4	103	9.1
231	0.7	100	3.3
219	0.8	99	8.1
218	0.5	86	1.8
217	0.4	83	3.0
207	1.5	82	1.3
201	2.6	81	1.5
185	1.0	79	2.4
184	0.7	70	4.2
160	5.2	57	4.2
159	36.4	45	3.8
145	15.2	44	3.8
142	12.1	43	100.0
129	10.1	42	4.1
117	12.1		

[a]Mass spectra from the xylobiose and 2',3',4'-tri-O-methyl-xylobiose systems are identical within operating variations of the mass spectrometer.

[b]Reactive intermediate from the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose in 0.1M sodium hydroxide at 30°C.

TABLE XVI

MASS SPECTRAL DATA FOR 4-DEOXY-2,3-PENTODIULOSE REDUCED WITH SODIUM BORODEUTERIDE (ACETATE DERIVATIVE)[a,b]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
248	1.7	103	10.7
247	2.4	101	4.2
233	0.6	100	10.7
219	1.3	85	3.2
207	2.0	84	3.8
203	1.7	83	2.2
187	1.3	73	1.5
186	1.7	72	4.4
161	4.9	71	1.6
160	45.2	62	2.2
147	6.3	61	2.0
146	6.0	60	2.4
145	13.0	58	7.1
144	9.5	57	3.2
143	5.6	56	2.7
131	17.9	55	1.4
118	19.0	52	1.4
114	3.3	51	1.9
105	2.0	45	4.1
104	3.2	43	100.0

[a]Mass spectra from the xylobiose and 2',3',4'-tri-O-methyl-xylobiose systems are identical within operating variations of the mass spectrometer.

[b]Reactive intermediate from the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose in 0.1M sodium hydroxide at 30°C.

APPENDIX IV

PRODUCT IDENTIFICATION

Gas-liquid chromatography-mass spectrometry (GLC-MS) was the major tool used in identifying the per-O-trimethylsilyl (TMS) derivatives of the products from the alkaline degradations. In the discussion of the GLC-MS results, the chemical names refer to the underivatized form of the product. The product numbers refer to compounds reported in Table III and IV, and to peaks in the gas chromatograms shown in Fig. 13 and 14. Reaction products common to xylobiose and 2',3',4'-tri-O-methyl-xylobiose reactions had very similar GLC-MS spectra varying only in the relative intensities of the peaks. Consequently, only a representative spectrum is provided for each compound. In most cases, a reference spectrum from a literature source is included for comparison. GLC and mass spectrometer conditions are reported in the Experimental section.

Xylobiose and 2',3',4'-tri-O-methyl-xylobiose Product 1 was identified as lactic acid. The mass spectrum of lactic acid is shown in Fig. 35 along with a reference spectrum (74). The spectrum of lactic acid is characterized by a parent-15 (M-15) ion at m/e 219 which loses trimethylsilanol (TMSiOH) to form the fragment at m/e 129. A significant ion at m/e 117 results from cleavage of the C-1--C-2 bond as shown below. The M-15 ion rearranges and loses carbon monoxide to form the fragment at m/e 191 as shown in Fig. 36. The ion at m/e 191 loses acetaldehyde to form a fragment with m/e 147. The ion at m/e 190 has been suggested (75) to arise from loss of acetaldehyde from the parent ion although no mechanism for such a loss has been provided.

Product 2 from the degradation of xylobiose and 2',3',4'-tri-O-methyl-xylobiose was identified as glycolic acid. The mass spectrum of Product 2 and a

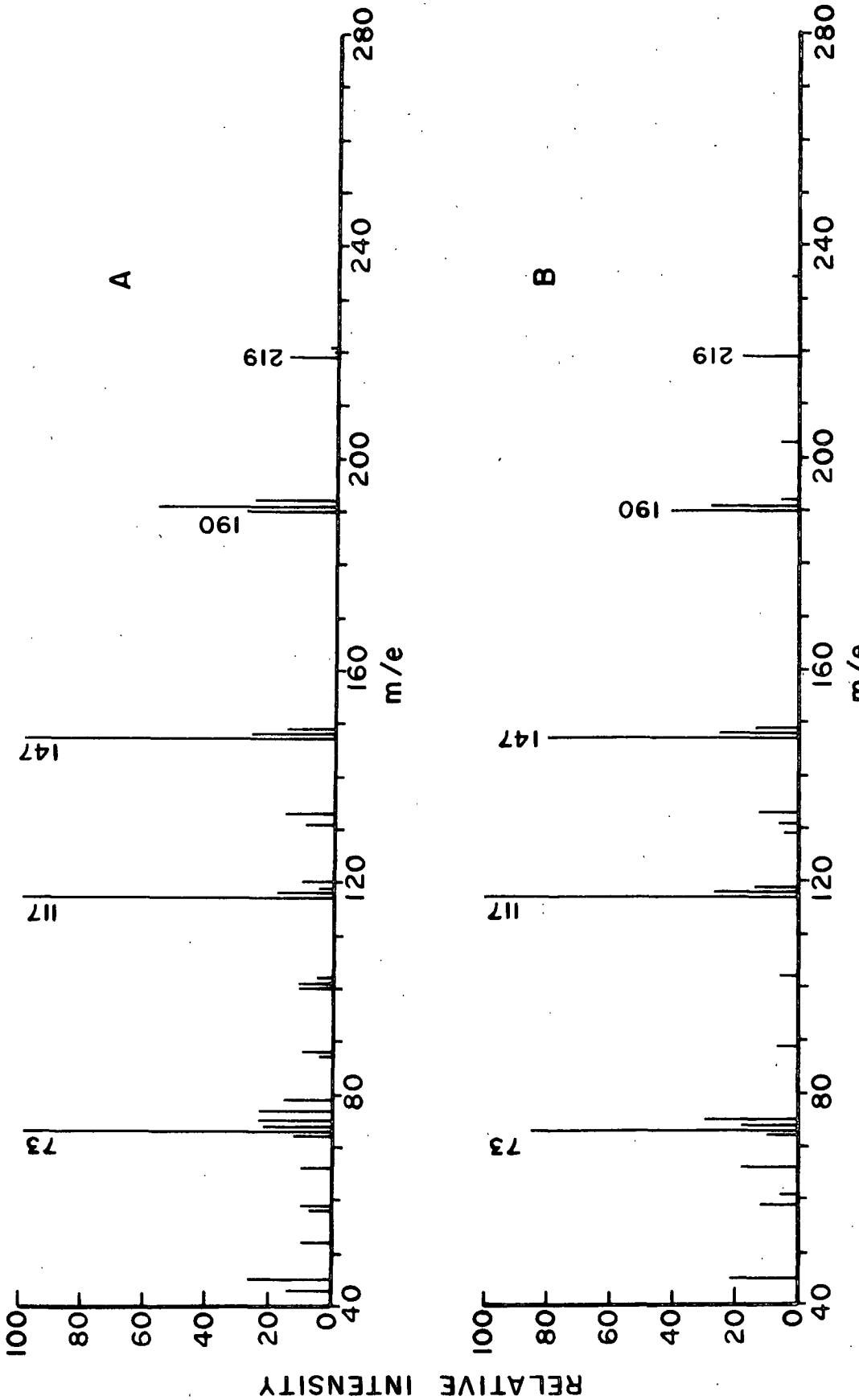


Figure 35. Mass Spectra (70eV) of the Trimethylsilyl Derivative of Lactic Acid; A-Xylobiose and 2',3',4'-Tri-O-Methyl-Xylobiose Product 1; B-Millard (74)

reference spectrum (74) are displayed in Fig. 37. The spectrum of Product 2 is characterized by an M-15 ion at m/e 205. The M-15 ion rearranges and loses carbon monoxide to form an ion at m/e 177 as shown in Fig. 38. Loss of formaldehyde from the ion at m/e 177 provides a fragment at m/e 147. Cleavage of the C-1 - C-2 bond results in fragments at m/e 117 and m/e 103 as shown below.

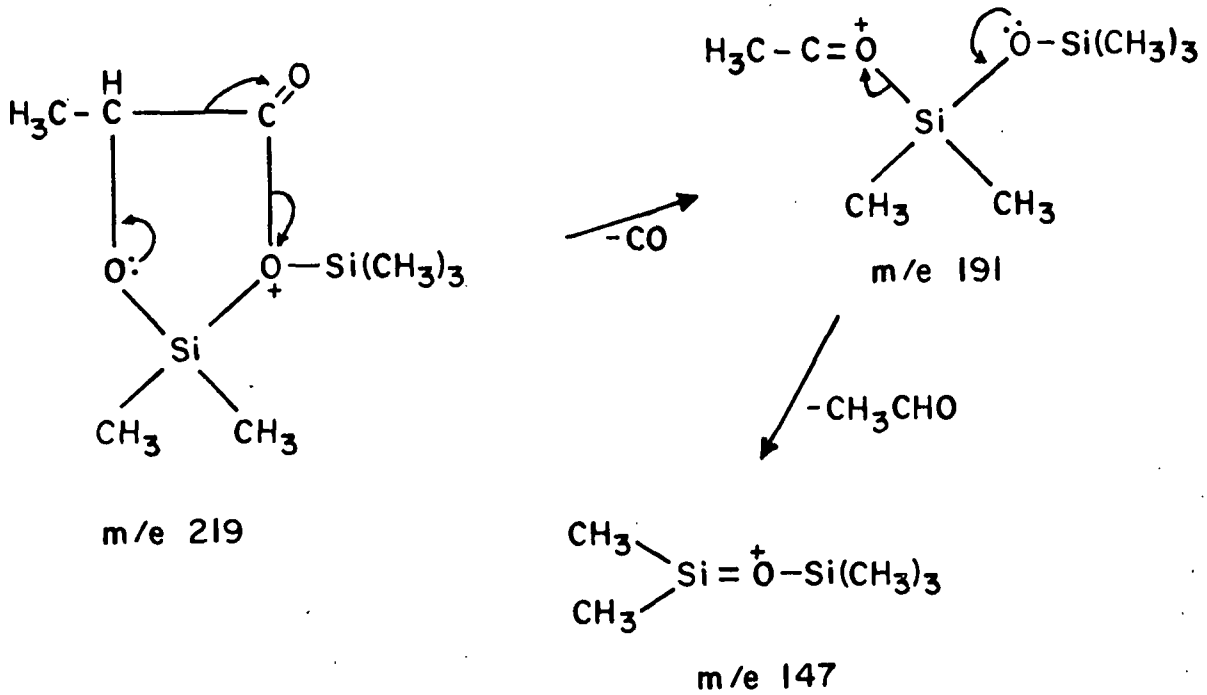
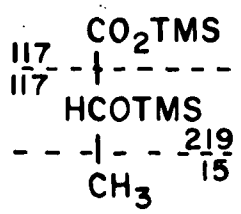


Figure 36. Fragmentation of M-15 Ion of Lactic Acid (75)



M 234

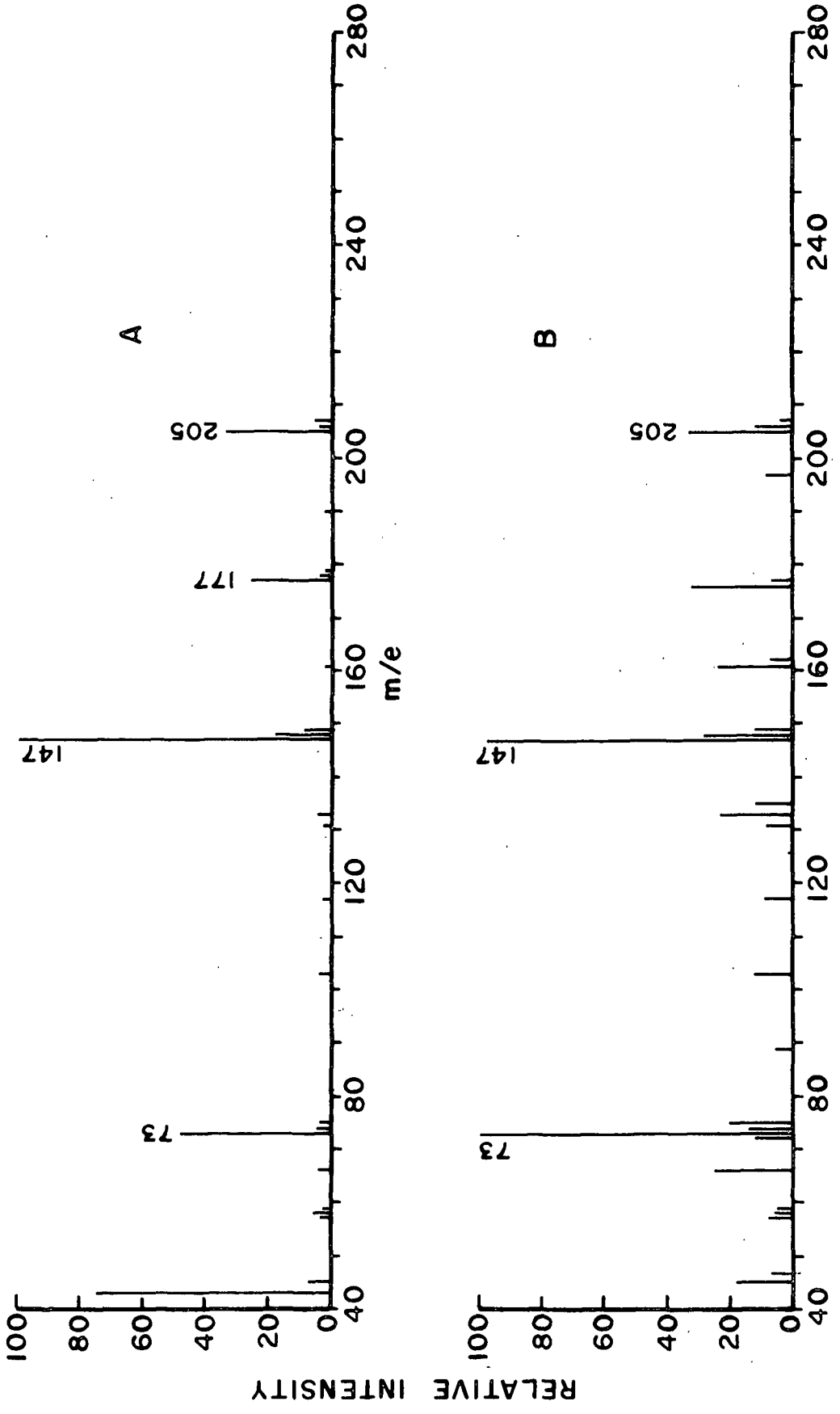
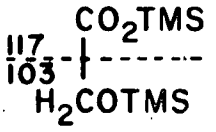


Figure 37. Mass Spectra (70ev) of the Trimethylsilyl Derivative of Glycolic Acid: A-Xylobiose and 2',3',4'-Tri-O-Methyl-xylobiose Product 2; B-Millard (74)



M 220

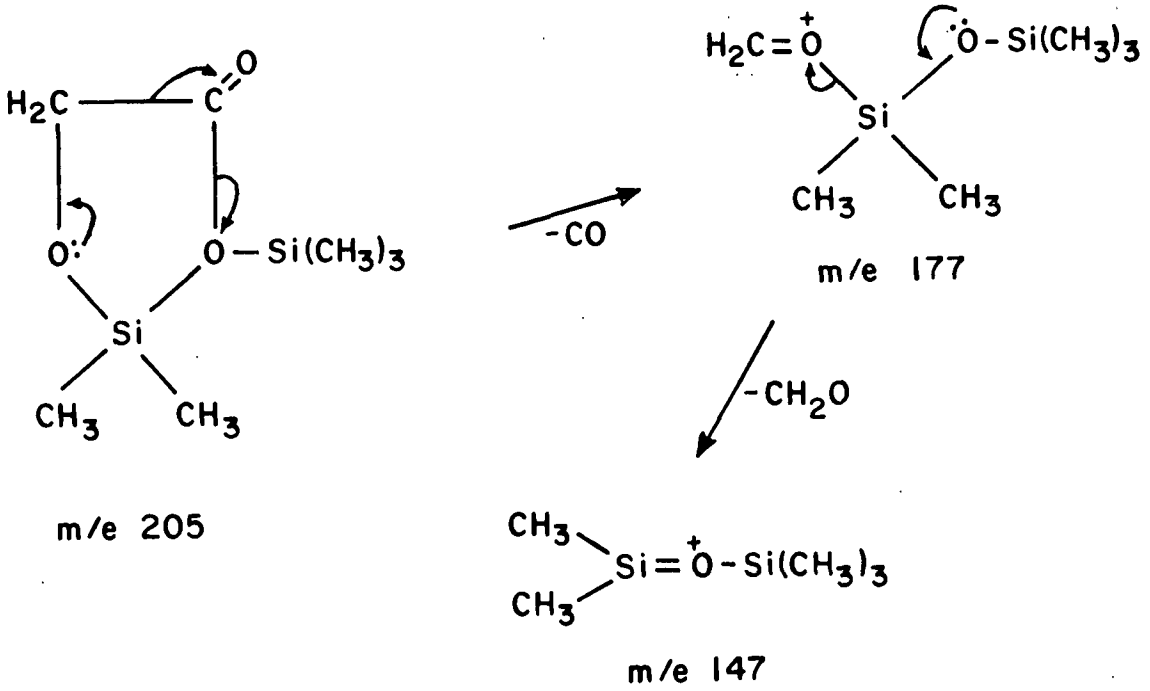
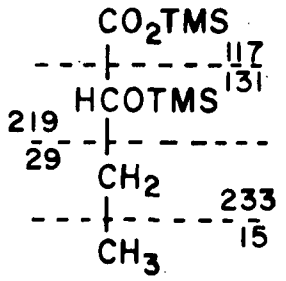


Figure 38. Fragmentation of M-15 Ion of Glycolic Acid (75)

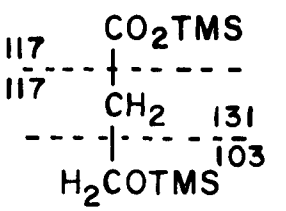
Xylobiose and 2',3',4'-tri-O-methyl-xylobiose Product 3 was identified as 2-hydroxybutyric acid. The spectrum of Product 3 and a reference spectrum are provided in Fig. 39. The spectrum of Product 3 is characterized by an ion at m/e 233 resulting from loss of a methyl group from the parent ion (M-15). Similarly, loss of TMSiOH from the parent ion provides a fragment at m/e 143.

The parent ion also loses carbon monoxide (as shown in Fig. 40) to form a fragment at m/e 205. Subsequent loss of propionaldehyde from the m/e 205 ion provides a fragment at m/e 147. Cleavage of the C-1 - C-2 bond shown below provides ions at m/e 117 and m/e 131. Another important ion at m/e 219 results from cleavage of the C-2 - C-3 bond.



M 248

Xylobiose and 2',3',4'-tri-O-methyl-xylobiose Product 4 was identified as 3-hydroxypropionic acid. The spectrum of Product 4 and the authentic compound are provided in Fig. 41. The spectrum of Product 4 contains only one peak in the high mass region at m/e 219 resulting from the loss of a methyl group from the parent ion (M-15). Subsequent loss of TMSiOH from the M-15 peak accounts for an ion at m/e 129. The peaks that normally result from cleavage of the C-1 - C-2 and C-2 - C-3 bonds, as shown below, are either weak or absent, indicating an unpreferred fragmentaion about the methylene carbon (75). The intense ions at m/e 177 and m/e 147 result from a series of decomposition reactions of the M-15 ion as shown in Fig. 42.



M 234

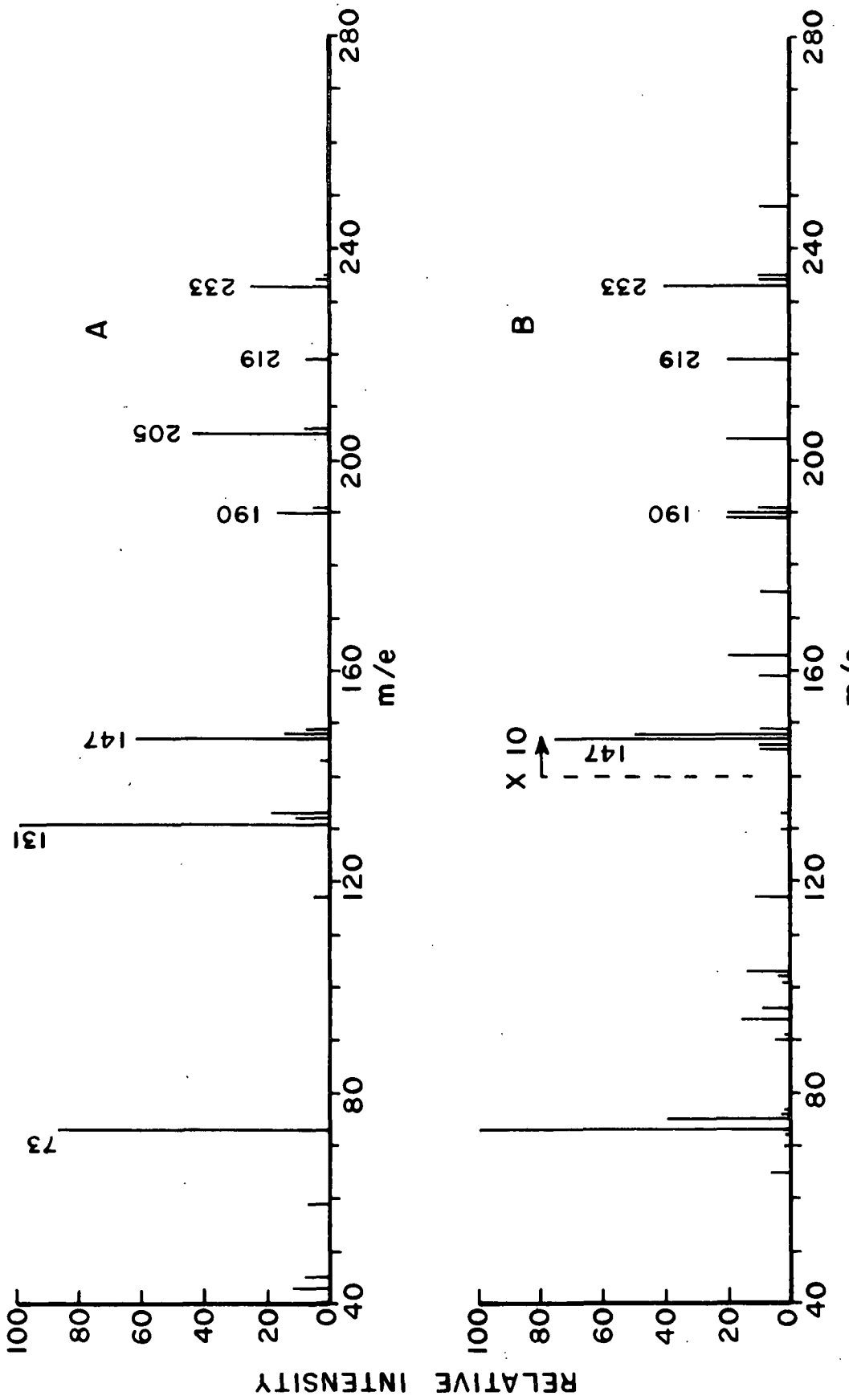


Figure 39. Mass Spectra (70ev) of Trimethylsilyl Derivative of 2-Hydroxybutyric Acid: A-Xylobiose and 2',3',4'-Tri-O-Methyl-Xylobiose Product 3; E-Millard (74)

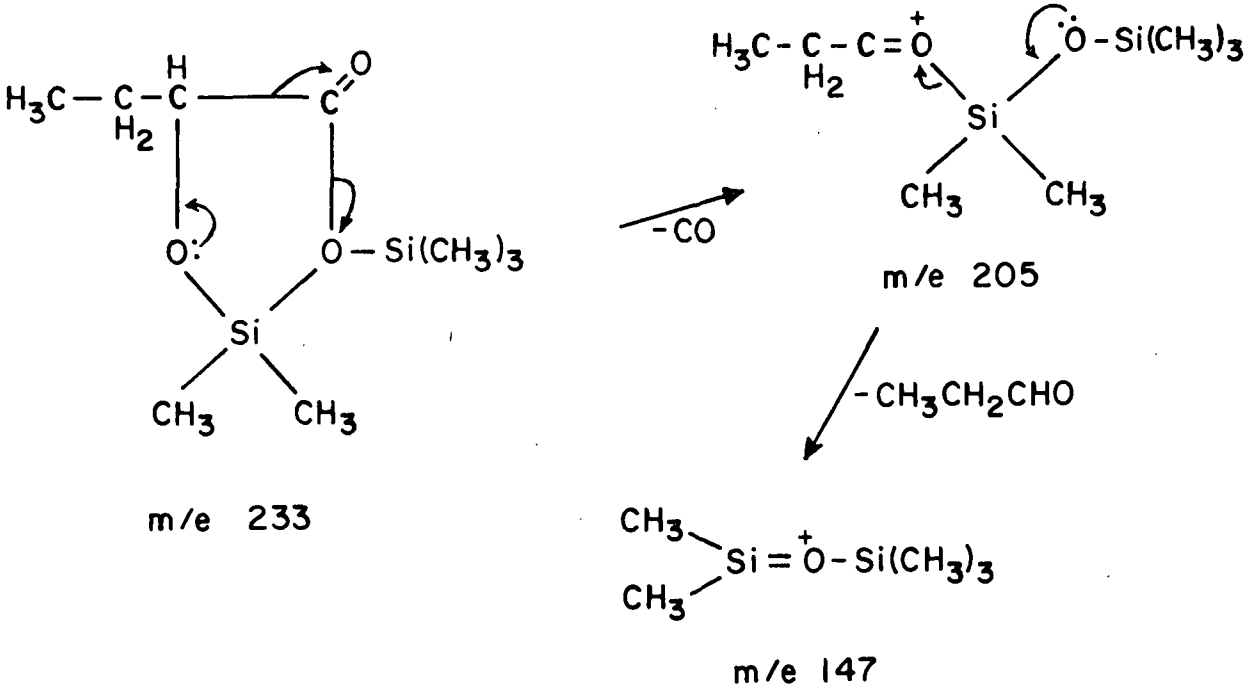
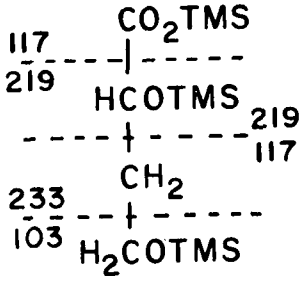


Figure 40. Fragmentation of M-15 Ion of 2-Hydroxybutyric Acid (75)

Product 5 from the Xylobiose and 2',3',4'-tri-O-methyl-xylobiose reactions was identified as 2,4-dihydroxybutyric acid. The spectrum of Product 5 correlates well with the reference spectrum as shown in Fig. 43. The main peak in the upper mass region is at m/e 321 and arises from the loss of a methyl group from the parent ion (M-15). The ion at m/e 219 can result, as shown below, from either C-1 - C-2 or C-2 - C-3 bond cleavage. A potential source for the ion at m/e 147 is decomposition of the M-15 ion shown in Fig. 44.



M 336

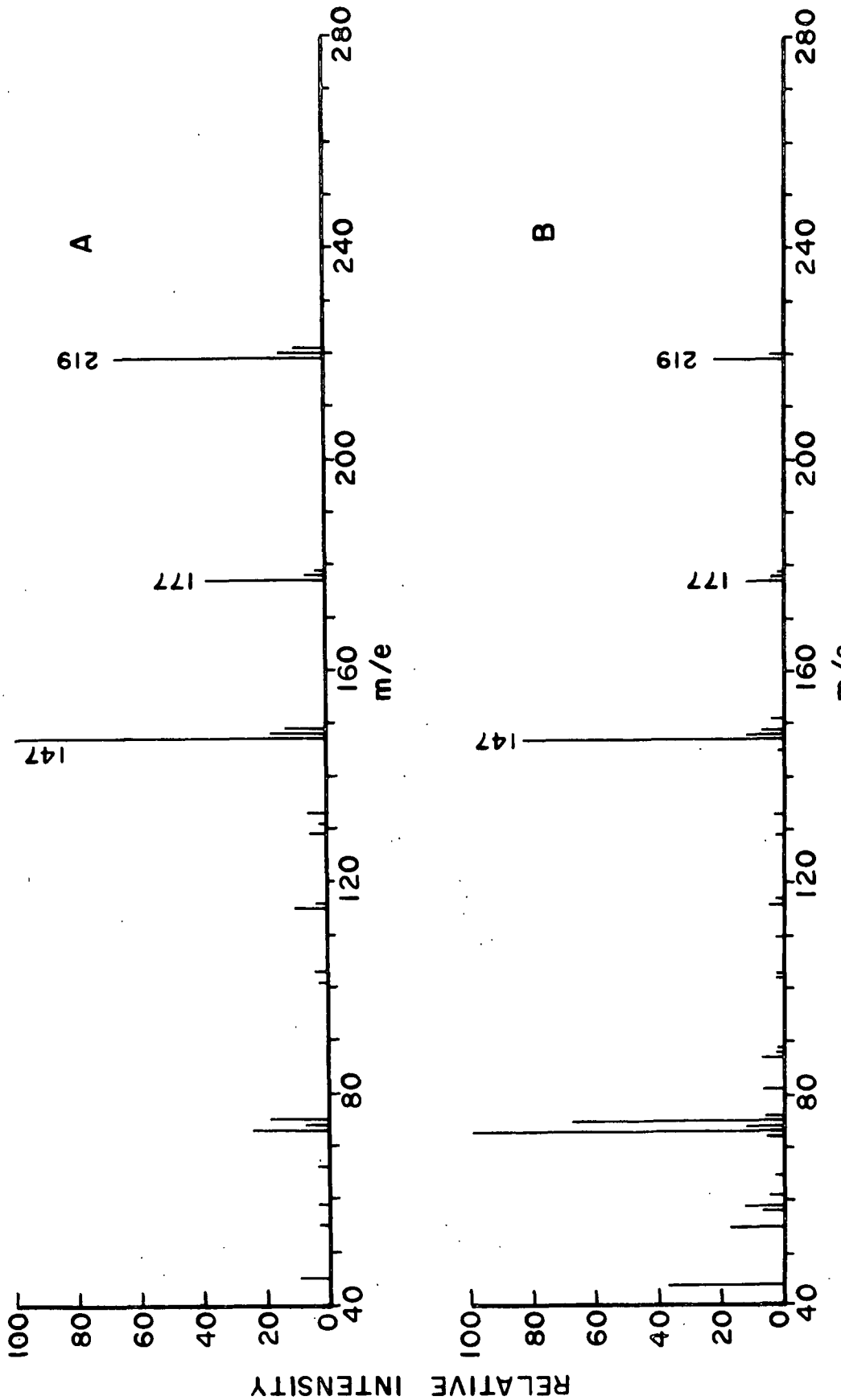


Figure 41. Mass Spectra (70ev) of Trimethylsilyl Derivative of 3-Hydroxypropionic Acid: A-Xylobiose and 2',3',4'-Tri-O-Methyl-Xylobiose Product 4; B-Millard (74)

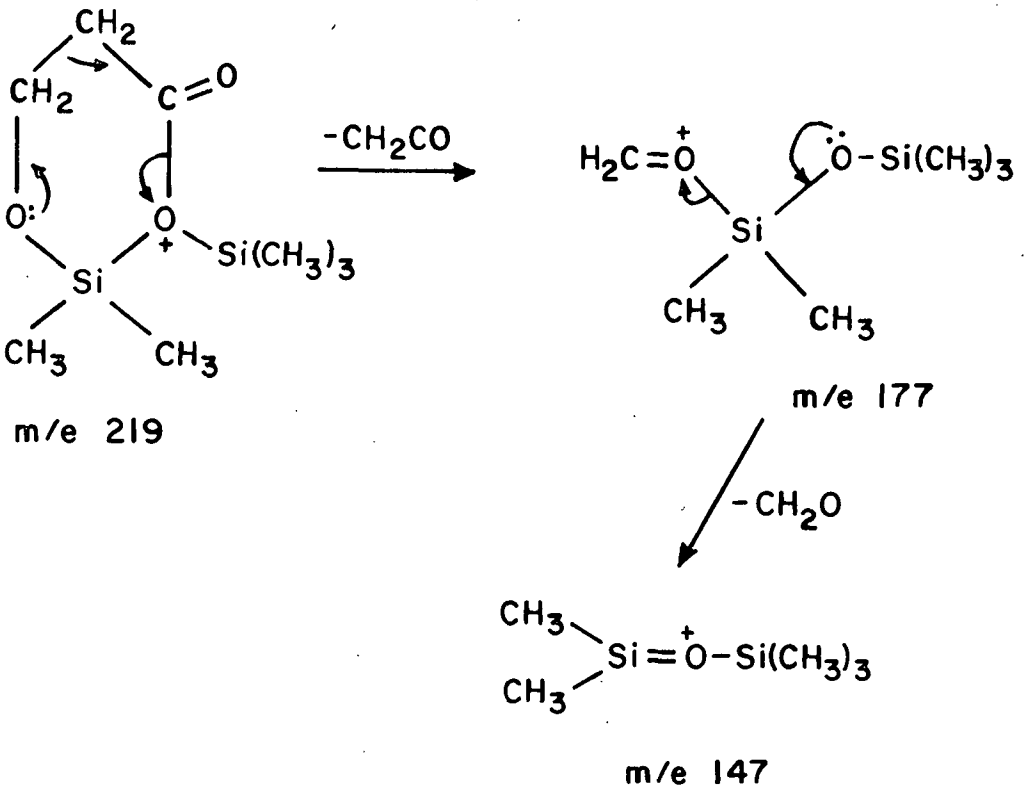


Figure 42. Fragmentation of M-15 Ion of 3-Hydroxypropionic Acid (75)

Xylobiose and 2',3',4'-tri-O-methyl-xylobiose Product 6 was identified as D-xyloisosaccharinic acid. The spectrum of Product 6 correlates well with the spectrum of the authentic sample as shown in Fig. 45. The spectrum of Product 6 is characterized by a peak at m/e 423 due to loss of a methyl group from the parent ion (M-15). Subsequent loss of TMSiOH from the M-15 ion results in a fragment at m/e 333. The ions at m/e 335 and m/e 103 result from cleavage of the side chain at C-2 rather than C-3 - C-4 cleavage (75). Cleavage of the C-1 - C-2 bond, as shown below, results in a fragment at m/e 321 which loses TMSiOH to form an intense ion at m/e 231. In addition, an M-30 ion at m/e 408 is formed after loss of formaldehyde from the parent ion via a MacLafferty type rearrangement shown in Fig. 46.

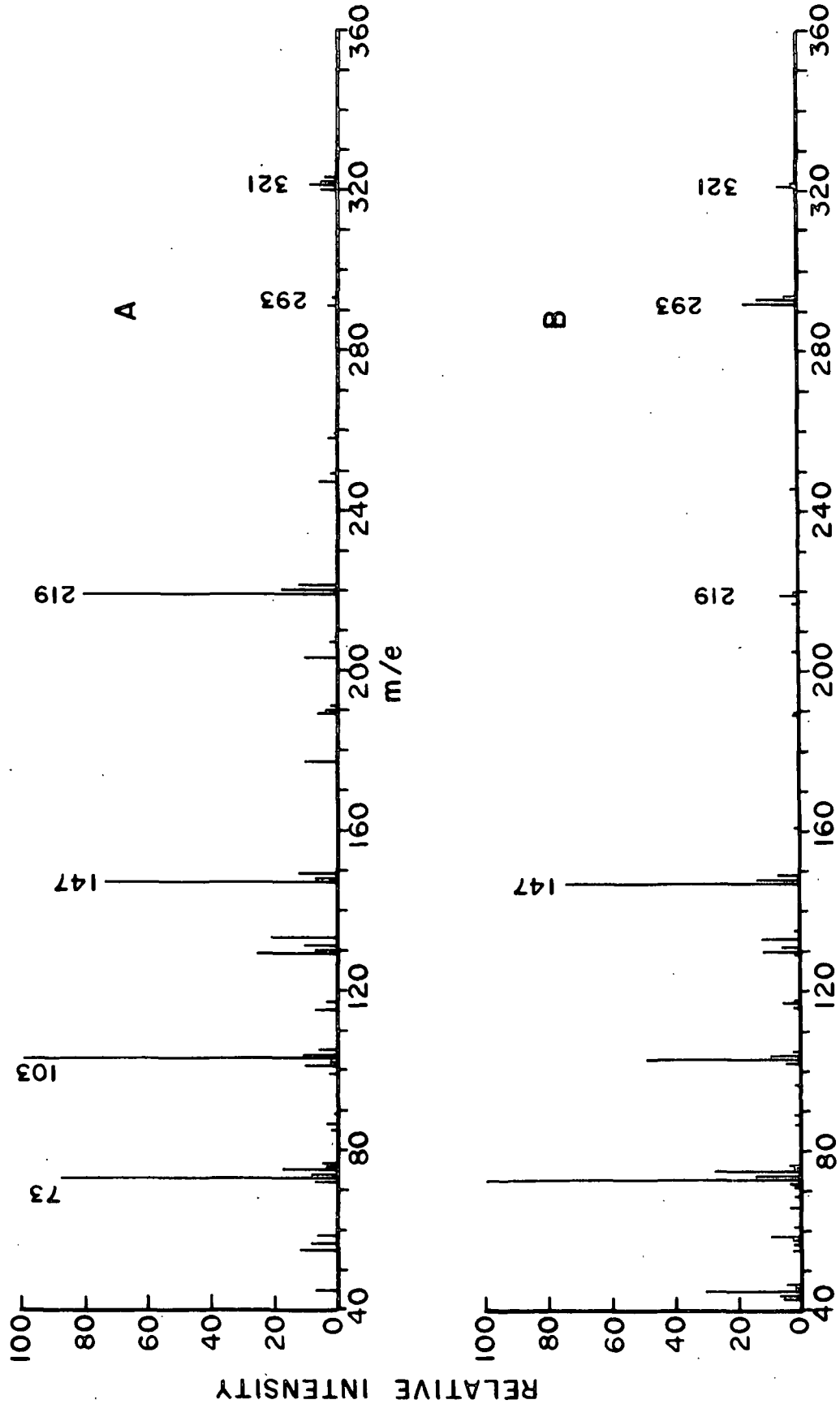
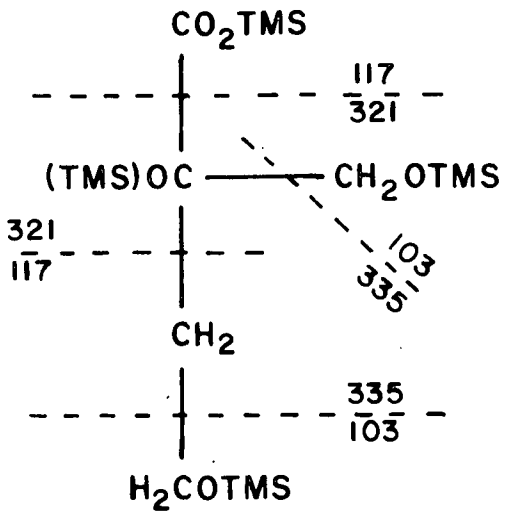


Figure 43. Mass Spectra (70ev) of Trimethylsilyl Derivative of 2,4-Dihydroxybutyric Acid: A-Xylobiose and 2',3',4'-Tri-O-Methyl-Xylobiose Product 5; B-Millard (74)



M 438

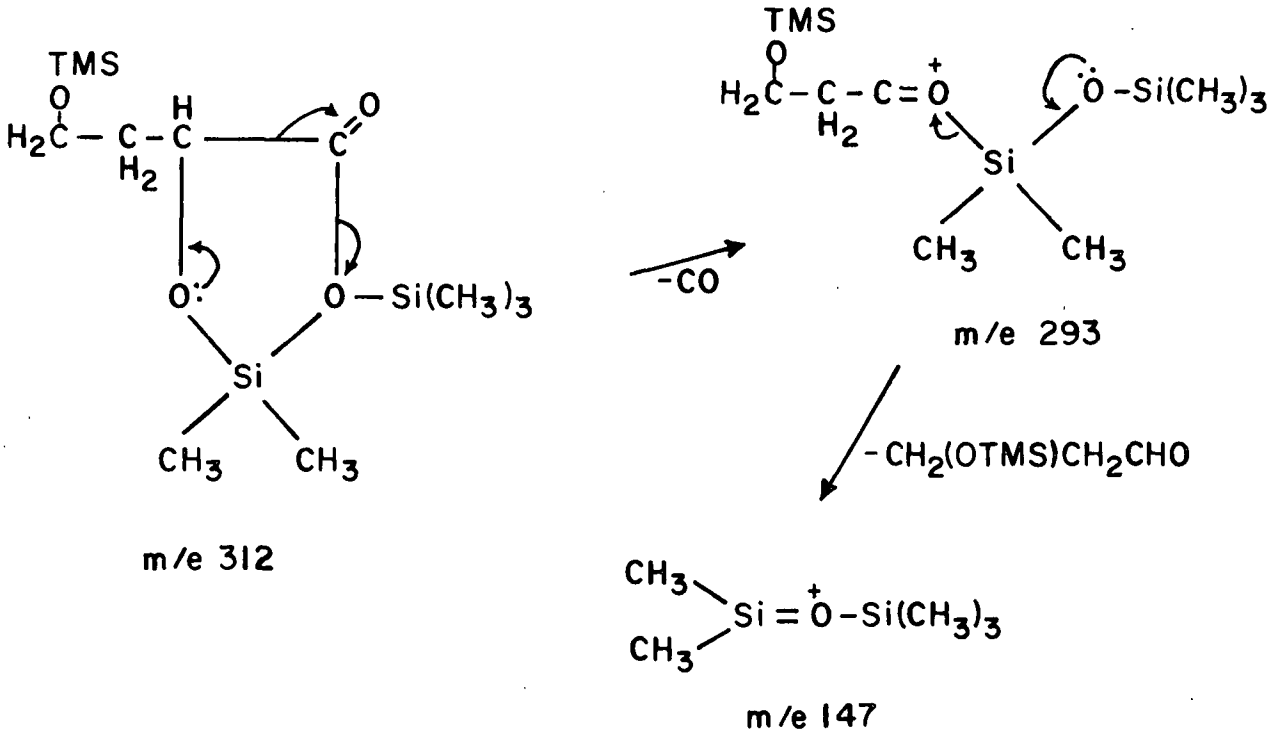


Figure 44. Fragmentation of M-15 Ion of 2,4-Dihydroxybutyric Acid (75)

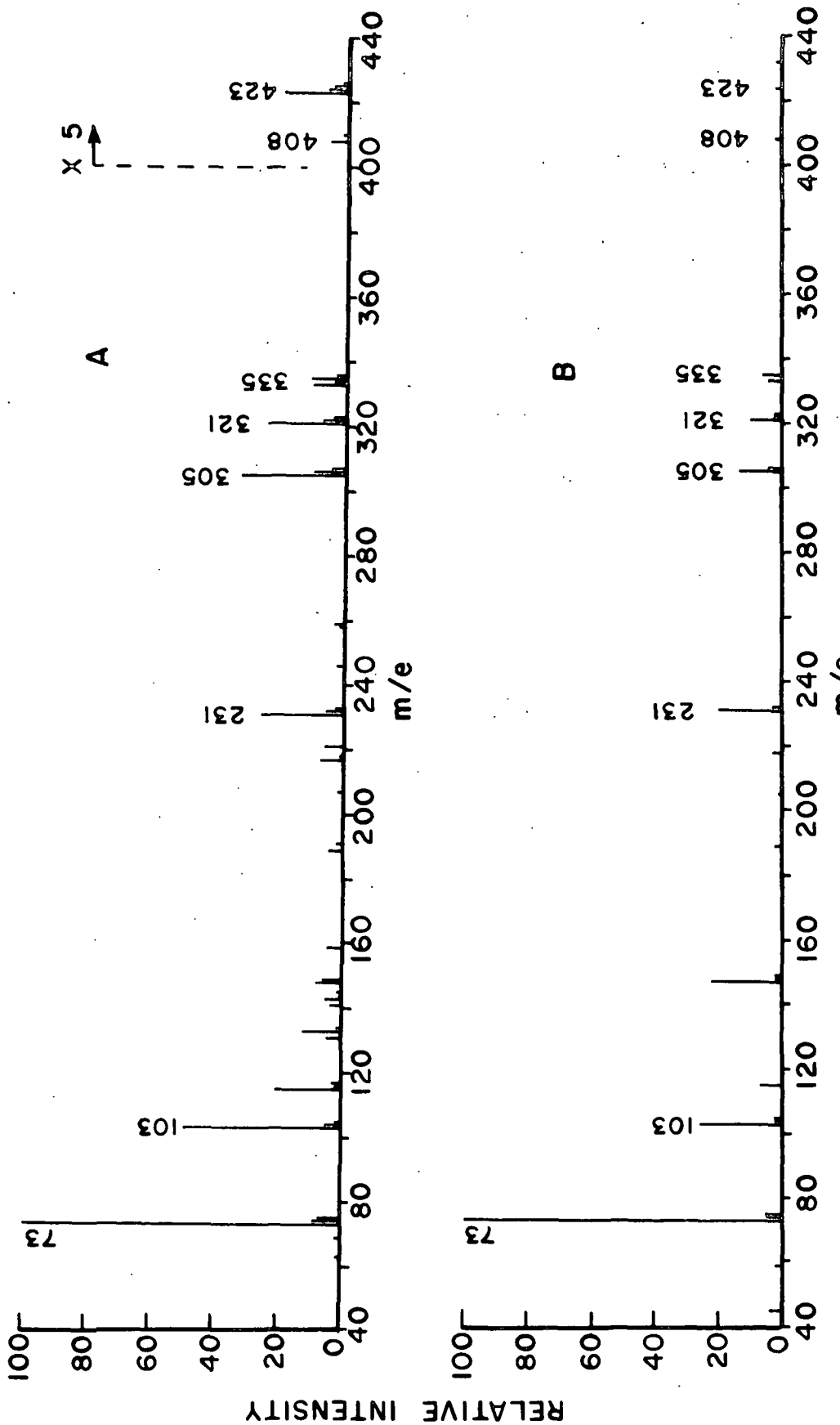
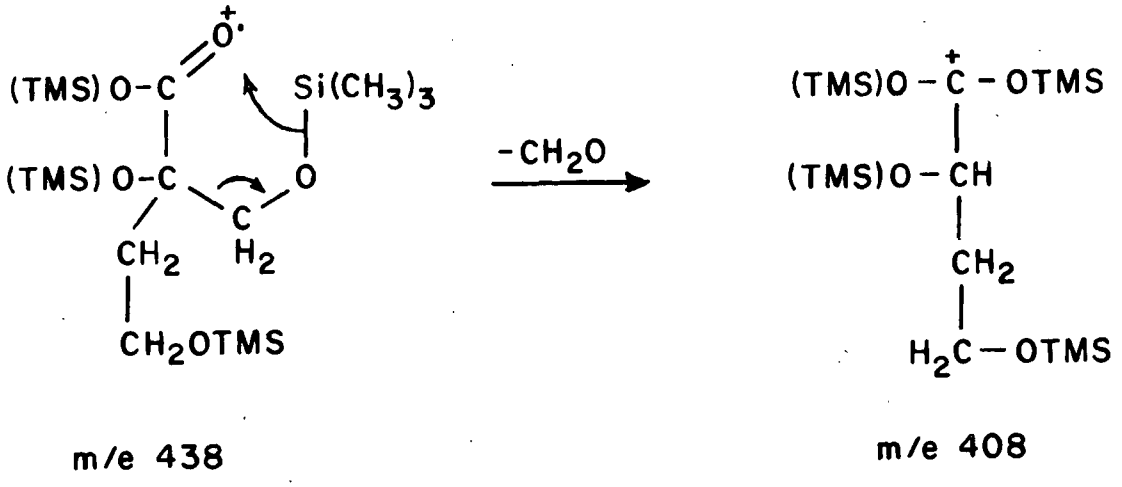


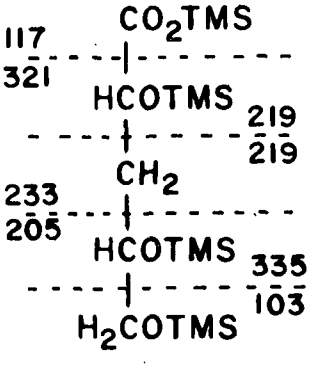
Figure 45. Mass Spectra (70ev) of Trimethylsilyl Derivative of D-Xyloisosaccharinic Acid: A-Xylobiose and 2',3',4'-Tri-O-Methyl-Xylobiose Product 6; B-Peterson (75)



m/e 438 m/e 408

Figure 46. Rearrangement of Parent Ion of D-Xyloisosaccharinic Acid via MacLafferty Rearrangement (75)

Xylobiose Products 7 and 8 were identified as isomeric 3-deoxy-pentonic acids. The mass spectra of these isomers were essentially identical. Therefore, a representative spectrum of Product 7 and 8, and a spectrum from the literature are shown in Fig. 47. The mass spectra of Products 7 and 8 are characterized by a M-15 ion at m/e 423 which loses TMSiOH to form a fragment at m/e 333. The ion at m/e 335 results from C-4 - C-5 cleavage, and subsequent loss of TMSiOH from this ion results in an intense peak at m/e 245. Similarly, cleavage of the C-1 - C-2 bond provides an ion at m/e 321 which loses TMSiOH to form an intense fragment at m/e 231. The ion at m/e 205 results from cleavage of the C-3 - C-4 bond.



M 438

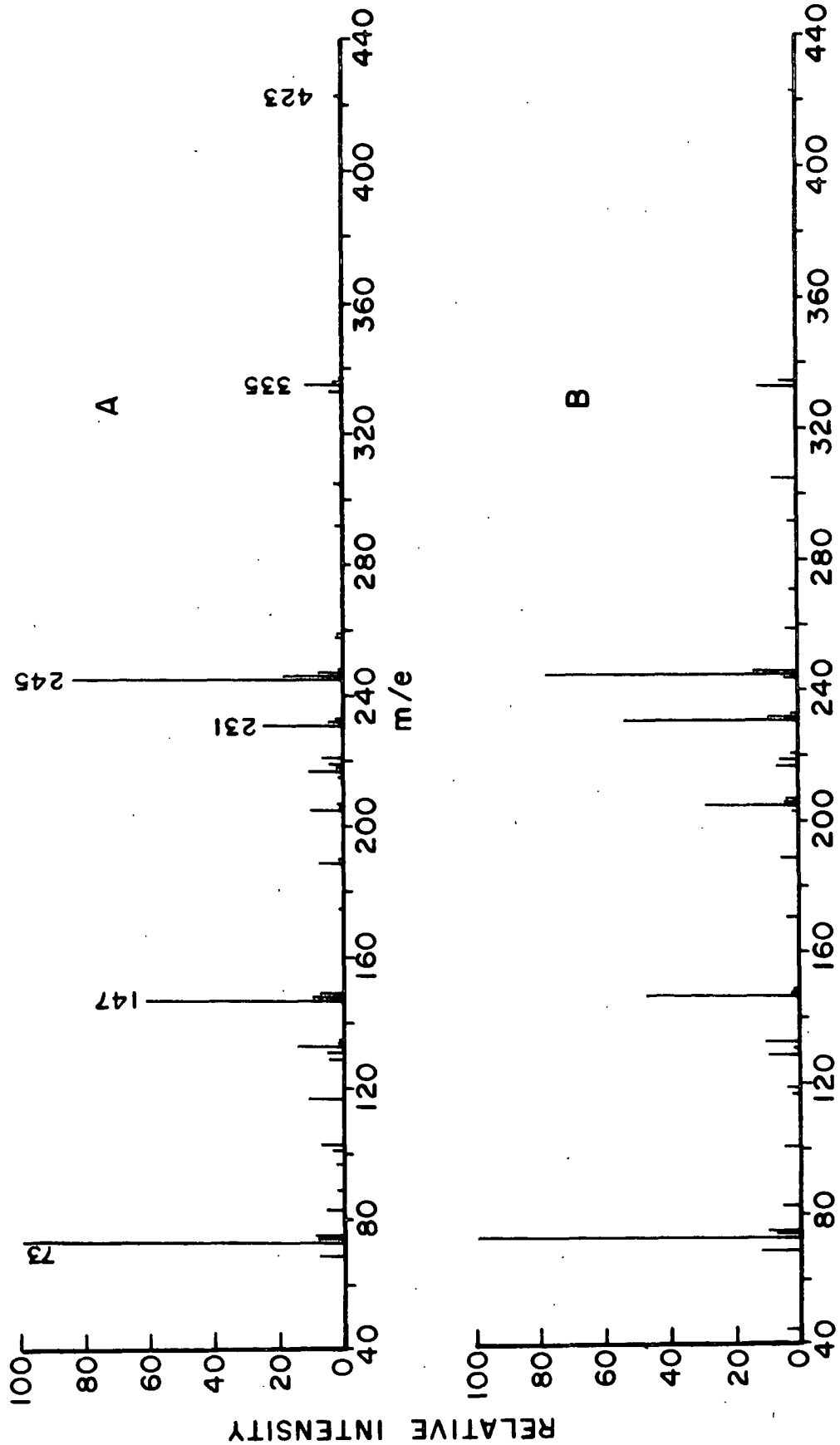


Figure 47. Mass Spectra (70ev) of Trimethylsilyl Derivative of 3-Deoxypentonic Acid: A-Xylobiose Product 7 and 8; B-MacLeod (35)

The remaining Products (9-14) from the degradation of 2',3',4'-tri-O-methyl-xylobiose are attributed to the nonreducing end of the molecule. Products 9-12 appear to be isomeric compounds, but were not identified. A sample spectrum of Product 9-12 from the 2',3',4'-tri-O-methyl-xylobiose and 2,3,4-tri-O-methyl-D-xylose degradations are presented in Fig. 48.

Products 13 and 14 are also common to the reactions of 2',3',4'-tri-O-methyl-xylobiose and 2,3,4-tri-O-methyl-D-xylose. A sample spectrum of each product is shown in Fig. 49. The identity of these compounds is not known.

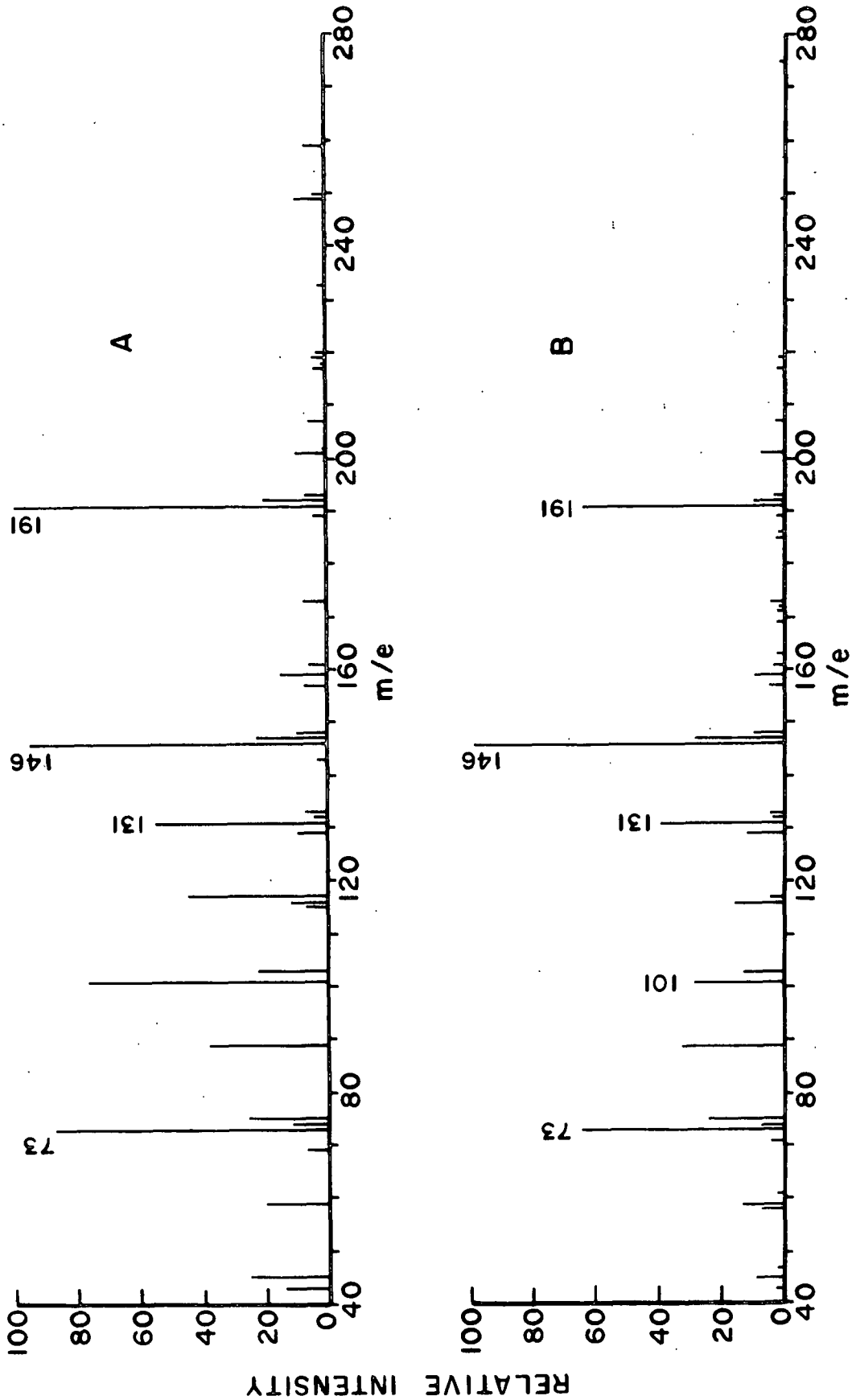


Figure 48. Mass Spectra (70ev) of Trimethylsilyl Derivative of Unknown Product: A-2',3',4'-Tri-O-Methyl-Xylobiose Product 9-12; B-2,3,4-Tri-O-Methyl-Xylose Product 9-12

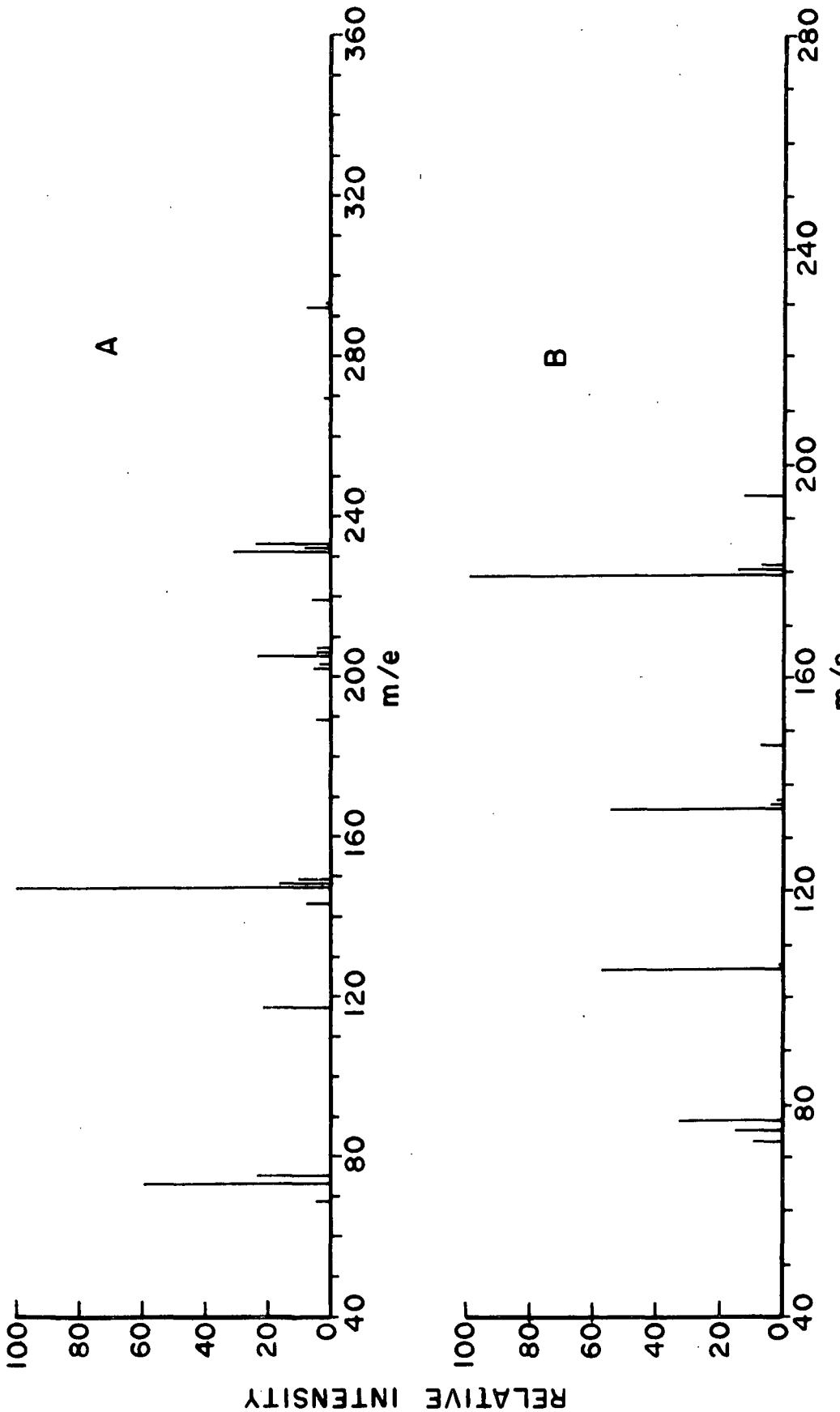


Figure 49. Mass Spectra (70ev) of Trimethylsilyl Derivative of 2',3',4'-Tri-O-Methyl-Xylobiose and 2,3,4-Tri-O-Methyl-Xylose Unknown Product 13 (A) and 14 (B)

APPENDIX V

MASS SPECTRAL DATA

The following appendix contains the mass spectral data for the products from the degradation of xylobiose, 2',3',4'-tri-O-methyl-xylobiose, D-xylose, and 2,3,4-tri-O-methyl-D-xylose.

TABLE XVII

MASS SPECTRAL DATA FOR LACTIC ACID (TMS DERIVATIVE)[a] (XYLOBIOSE AND XYLOSE PRODUCT 1)

m/e	Relative Abundance, %	m/e	Relative Abundance, %
221	0.7	118	6.9
220	1.1	117	66.7
219	7.3	103	1.2
192	3.8	102	2.2
191	20.0	101	2.6
190	13.3	100	1.7
149	7.6	89	3.6
148	16.7	88	2.0
147	100.0	75	13.3
133	11.6	74	6.7
131	5.8	73	66.7
129	3.6	45	10.0
119	3.3	43	13.3

[a]Mass spectra from the xylose and xylobiose systems are identical within operating variations of the mass spectrometer.

TABLE XVIII

MASS SPECTRAL DATA FOR GLYCOLIC ACID (TMS DERIVATIVE) (XYLOBIOSE AND 2',3',4'-TRI-O-METHYL-XYLOBIOSE PRODUCT 2)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
207	6.7	134	3.7
206	11.9	133	13.4
205	40.3	117	9.0
192	0.4	103	5.2
191	0.7	95	2.4
190	2.7	88	6.0
179	3.0	81	6.0
178	5.2	75	13.4
177	25.4	75	11.9
162	3.0	73	74.6
161	9.0	66	14.9
149	16.4	59	9.0
148	25.4	58	8.2
147	100.0	45	9.0

[a]Mass spectra from the xylobiose and 2',3',4'-tri-O-methyl-xylobiose systems are identical within operating variations of the mass spectrometer.

TABLE XIX

MASS SPECTRAL DATA FOR 2-HYDROXYBUTYRIC ACID (TMS DERIVATIVE) (XYLOBIOSE AND 2',3',4'-TRI-O-METHYL-XYLOBIOSE PRODUCT 3)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
235	2.5	147	62.5
234	4.6	143	3.7
233	25.8	133	19.2
219	8.3	132	11.2
206	8.7	131	100.0
205	43.7	117	5.8
191	5.8	73	87.5
190	17.5	59	7.1
149	8.3	45	8.7
148	15.4	43	12.1

[a]Mass spectra from the xylobiose and 2',3',4'-tri-O-methyl-xylobiose systems are identical within operating variations of the mass spectrometer.

TABLE XX

MASS SPECTRAL DATA FOR 3-HYDROXYPROPIONIC ACID (TMS DERIVATIVE) (XYLOBIOSE AND 2',3',4'-TRI-METHYL-XYLOBIOSE PRODUCT 4)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
221	9.5	116	4.1
220	14.6	115	11.1
219	66.7	103	4.9
179	3.8	102	2.3
178	7.0	101	3.6
177	38.1	75	19.0
149	14.0	74	7.9
148	18.1	73	24.4
147	100.0	66	4.5
133	6.7	59	3.9
131	2.5	55	3.2
129	6.0	45	9.5

[a]Mass spectra from the xylobiose and 2',3',4'-tri-O-methyl-xylobiose systems are identical within operating variations of the mass spectrometer.

TABLE XXI

MASS SPECTRAL DATA FOR 2,4-DIHYDROXYBUTYRIC ACID (TMS DERIVATIVE) (XYLOSE, XYLOBIOSE AND 2',3',4'-TRI-O-METHYL-XYLOBIOSE PRODUCT 5)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
323	0.6	131	6.6
322	1.3	130	4.7
321	3.2	129	7.8
294	0.5	119	1.2
293	1.7	118	2.8
292	1.0	116	1.7
291	1.2	115	2.3
263	0.6	108	0.6
252	0.6	105	6.4
251	0.6	104	14.2
250	1.6	103	100.0
222	1.0	101	2.9
221	5.4	100	2.1
220	12.4	99	0.8
219	38.3	95	0.8
218	15.8	93	0.9
207	0.7	89	1.9
204	2.7	88	0.9
203	2.7	87	1.8
188	2.8	86	6.1
178	0.8	85	0.8
177	3.7	83	0.5
175	1.3	77	0.5
174	3.0	76	0.6
161	0.6	75	6.2
160	0.5	74	5.2
159	0.6	73	61.5
150	0.8	70	1.0
149	4.9	66	0.6
148	4.6	61	2.3
147	30.0	59	3.5
145	0.5	58	1.2
135	0.9	55	2.7
134	1.0	51	0.5
133	7.7		

[a]Mass spectra from the xylobiose, xylose, and 2',3',4'-tri-O-methyl-xylobiose are identical within operating variations of the mass spectrometer.

TABLE XXII

MASS SPECTRAL DATA FOR D-XYLOISOSACCHARINIC ACID (TMS DERIVATIVE) (XYLOBIOSE AND 2',3',4'-TRI-O-METHYL-XYLOBIOSE PRODUCT 6)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
425	3.7	231	75.9
424	8.9	221	17.7
423	21.5	217	12.7
410	3.7	205	10.1
409	3.0	191	6.3
408	6.6	189	8.9
337	7.6	149	20.3
336	7.6	148	10.1
335	35.4	147	63.3
334	10.1	143	6.3
333	31.6	141	8.9
323	8.9	133	12.7
322	22.8	131	5.1
321	50.6	129	7.6
307	10.1	117	6.3
306	27.8	116	5.1
305	87.3	115	25.3
247	3.4	103	35.4
246	5.2	101	4.6
245	10.1	83	4.4
243	3.0	75	8.9
233	8.9	74	8.9
232	16.5	73	100.0

[a]Mass spectra from the xylobiose and 2',3',4'-tri-O-methyl-xylobiose systems are identical within operating variations of the mass spectrometer.

TABLE XXIII

MASS SPECTRAL DATA FOR D-XYLOMETASACCHARINIC ACID (TMS PRODUCT) (XYLOSE AND XYLOBIOSE PRODUCT 7 + 8)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
425	0.2	189	9.2
424	0.8	163	2.3
423	1.2	159	2.2
347	1.0	150	1.4
337	1.2	149	8.1
336	1.3	148	9.8
335	9.4	147	58.0
334	1.8	144	1.0
333	4.4	143	2.8
321	2.2	141	1.4
306	1.2	135	1.6
305	1.9	134	2.0
293	1.3	133	14.2
292	4.4	131	3.7
291	1.1	129	7.3
260	1.6	119	1.6
259	3.7	117	9.6
258	4.3	116	2.2
247	5.6	115	1.1
246	15.4	103	10.5
245	72.2	102	2.4
243	3.3	101	4.9
233	1.9	99	1.3
232	3.9	97	5.4
231	25.4	93	1.0
229	1.0	89	2.1
223	1.3	83	6.0
221	4.9	78	2.7
219	3.4	77	2.6
218	1.2	75	11.5
217	9.1	74	7.9
215	1.9	73	100.0
207	2.7	69	10.0
206	2.4	63	1.2
205	10.2	59	1.8
201	1.0	58	2.2
191	3.3	56	0.9
190	1.6	55	1.3

[a]Mass spectra from the xylose and xylobiose reaction systems are identical within operating variations of the mass spectrometer.

TABLE XXIV

MASS SPECTRAL DATA FOR 2',3',4'-TRI-O-METHYL-XYLOBIOSE AND 2,3,4-TRI-O-METHYL-XYLOSE UNKNOWN PRODUCT 9-12 (TMS DERIVATIVE)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
275	2.5	144	3.5
260	1.7	143	19.4
259	1.4	133	14.3
249	5.1	132	6.1
235	2.5	131	40.8
233	6.5	129	5.1
232	3.4	117	2.6
219	8.2	116	15.3
217	12.2	105	6.1
203	3.5	103	23.5
202	4.2	101	41.8
201	11.2	93	6.1
193	9.2	89	42.9
192	12.2	75	26.5
191	71.4	74	10.2
189	3.4	73	71.4
175	5.3	72	6.1
173	3.5	71	6.1
163	3.1	69	7.1
161	6.1	61	7.1
159	10.2	60	2.7
148	5.1	59	24.5
147	30.6	58	8.2
146	100.0	55	8.2
145	7.1	45	18.4

[a]Mass spectra from the 2',3',4'-tri-O-methyl-xylobiose and and 2,3,4-tri-O-methyl-xylose are identical within operating variations of the mass spectrometer.

TABLE XXV

MASS SPECTRAL DATA FOR 2',3',4'-TRI-O-METHYL-XYLOBIOSE AND 2,3,4-TRI-O-METHYL XYLOSE UNKNOWN PRODUCT 13 (TMS DERIVATIVE)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
293	1.9	202	6.1
292	8.9	189	5.0
233	24.4	149	11.1
232	8.9	148	16.7
231	31.1	147	100.0
219	6.7	143	8.9
207	5.3	117	22.2
206	5.0	75	24.4
205	24.4	73	60.0
203	4.1	69	5.3

[a]Mass spectra from the 2',3',4'-tri-O-methyl-xylobiose and 2,3,4-tri-O-methyl-xylose reaction systems are identical within operating variations of the mass spectrometer.

TABLE XXVI

MASS SPECTRAL DATA FOR 2',3',4'-TRI-O-METHYL-XYLOBIOSE AND 2,3,4-TRI-O-METHYL-XYLOSE UNKNOWN PRODUCT 14 (TMS DERIVATIVE)[a]

m/e	Relative Abundance, %	m/e	Relative Abundance, %
194	13.1	135	54.8
181	7.1	107	1.3
180	15.5	106	1.9
179	100.0	105	57.1
147	7.1	77	33.3
137	2.2	75	15.5
136	4.4	73	9.5

[a]Mass spectra from the 2',3',4'-tri-O-methyl-xylobiose and 2,3,4-tri-O-methyl-xylose reaction systems are identical within operating variations of the mass spectrometer.